



# THE AMERICAN JOURNAL OF PATHOLOGY

*Official Publication of  
The American Association of Pathologists and Bacteriologists*

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VOLUME XXII

1946



ANN ARBOR  
MICHIGAN  
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PRINTED AT THE ANN ARBOR PRESS  
ANN ARBOR, MICHIGAN, U.S.A.

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# THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XXII

JANUARY, 1946

NUMBER 1

## PAGET'S DISEASE OF THE NIPPLE

WITH SPECIAL REFERENCE TO THE CHANGES IN THE DUCTS \*

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In 1936 I published a monograph<sup>1</sup> entitled "Paget's Disease of the Nipple and Its Relation to Surface Cancers and Precancerous States in General." Since then I have examined other examples and, while the results in the main have been confirmatory of my previous opinions, they call for certain modifications of my earlier views.

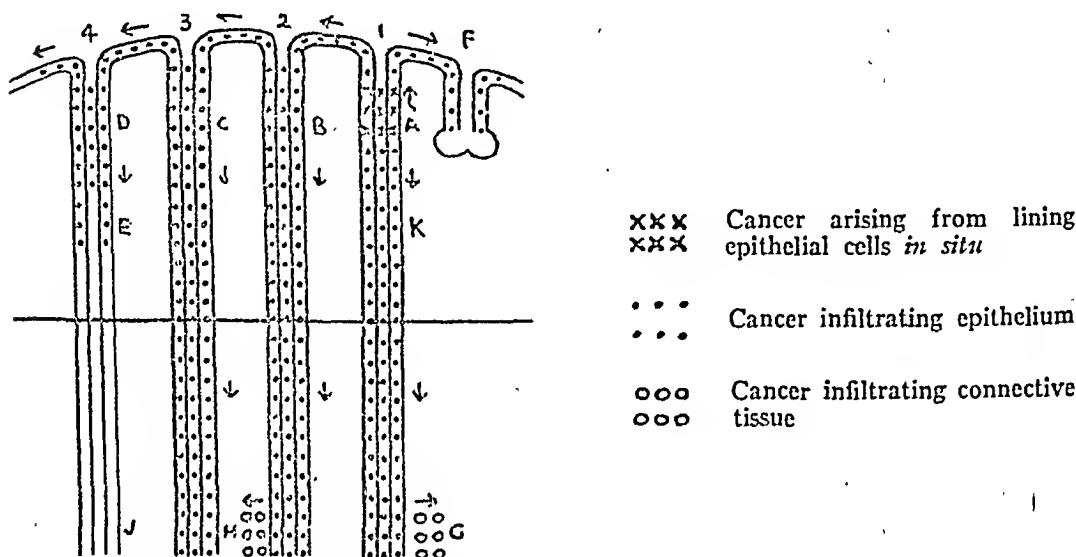
### THE NATURE, ORIGIN, AND MODE OF SPREAD OF PAGET'S DISEASE

My opinions under this heading are represented diagrammatically in Text-Figure 1. Paget's disease is neoplastic from the outset. The initial lesion is a duct cancer arising from lining epithelial cells *in situ*. This primary growth arises in one or more ducts near their outlets (Text-Fig. 1, duct 1,A). This primary duct cancer extends from its site of origin (Text-Fig. 1, duct 1,A) by continuous intra-epithelial spread downwards in the epithelium lining the duct wall to the acini, and upwards and outwards into the epidermis where it causes the cutaneous lesion. From the intra-epithelial extension in the epidermis the cancer spreads down neighboring ducts (Text-Fig. 1, ducts 2, 3, and 4) and pilosebaceous follicles (Text-Fig. 1,F) as in the duct primarily affected. At a later stage the cancer which has spread down a duct into the underlying breast, either directly (Text-Fig. 1, duct 1) or indirectly by way of the epidermis (Text-Fig. 1, duct 2), breaks through the wall of the duct (Text-Fig. 1, duct 1,G, or duct 2,H) or acinus, and infiltrates the connective tissue of the breast. There is thus a link between the primary ductal growth (Text-Fig. 1, duct 1,A) and the infiltrating cancer (Text-Fig. 1, duct 1,G, or duct 2,H) in the underlying breast.

In Paget's disease the cancer spreads down the duct, not in the form of a solid column (as in permeation of lymphatic vessels) but as a hollow cylinder (Text-Fig. 1, duct 4,E); speaking generally, however, the cancer, as it spreads down the duct, disintegrates the lining epi-

\* Received for publication, March 16, 1945.

thelium of the duct and cancer cells occupy the lumen (Text-Fig. 1, ducts 1,K; 2,B; 3,C; and 4,D). In Paget's disease the duct cancer which results from centrifugal spread from the site of origin is a special variety and is essentially different from ordinary duct cancer. It is not suggested that there is anything special about the cancer cells; the special quality is in the intra-epithelial mode of spread. The epidermal lesion and the special duct cancer of Paget's disease are essentially the same; minor histological differences can be accounted for by variation in anatomical situation; both are examples of cancer spreading by the intra-epithelial route.



Text-Fig. 1. Diagrammatic representation of possible sequences of events in Paget's disease of the nipple. Continued in Text-Figures 2 to 4.

In Paget's disease the duct changes due to intra-epithelial spread may be simulated by a newgrowth arising from duct epithelium *in situ* when such a primary growth does not show the formation of papillomatous processes; further, just as Paget's disease of the skin of the nipple is simulated microscopically by cancer which has spread from the connective tissue of the breast directly into the epidermis (Text-Fig. 4,W), so the special duct cancer of Paget's disease is simulated when a cancer which is infiltrating the connective tissue of the breast breaks into ducts which, until then, have been free from growth (Text-Fig. 4, duct 3,T).

#### ORDINARY DUCT CANCER AND SPECIAL DUCT CANCER OF PAGET'S DISEASE

There are three outstanding differences between ordinary duct cancer and the special duct cancer of Paget's disease.

In the ordinary duct cancer: (1) The growth arises as a result of the proliferation of lining epithelial cells *in situ*. (2) The ducts show

a variety of changes, and all stages of transition may be seen from simple papillomatous growth to masses of undifferentiated cells, that is, from a precancerous to a cancerous state. (3) The neoplastic proliferation within the ducts may occur in multicentric foci of origin, not only in different ducts, but also in different parts of the same duct.

In the special duct cancer of Paget's disease: (1) The growth does not arise from the lining epithelial cells *in situ*; the cancer cells have infiltrated from a distance as a part of a centrifugal spread by the intra-epithelial route from the primary lesion. (2) The growth is one of full-fledged cancer; no precancerous or transitional stages are to be seen. (3) There are no separate foci of origin.

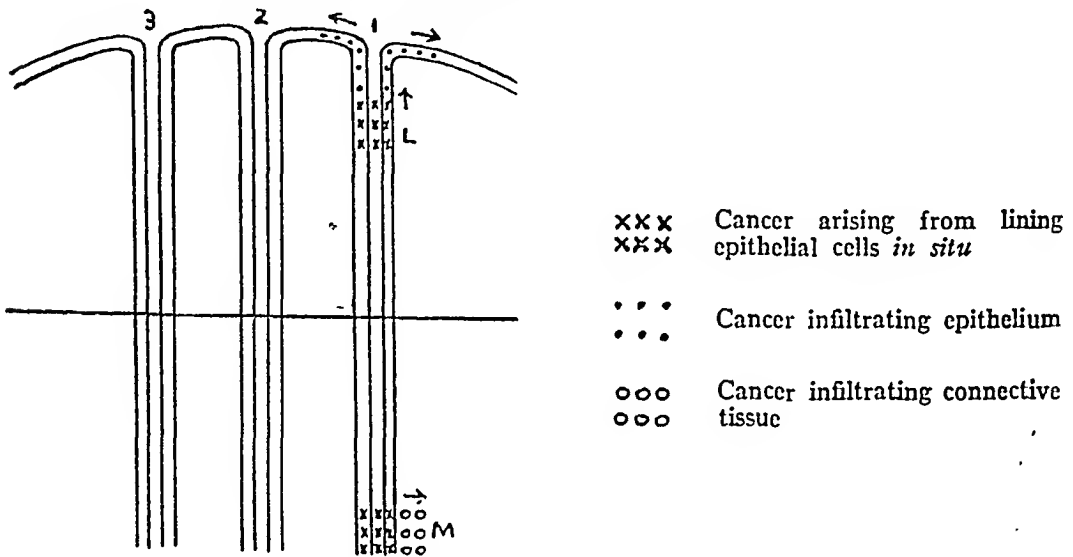
Duct cancer spreading by the intra-epithelial route (special duct cancer of Paget's disease) is not necessarily limited to Paget's disease, but it is the distinctive feature of the duct changes in Paget's disease, just as the intra-epithelial cancerous extension in the epidermis is the distinctive feature of the skin lesion in Paget's disease. In studying the early changes in the ducts in Paget's disease of the nipple great difficulty is experienced in distinguishing between those portions of the lesion in which there is neoplastic overgrowth *in situ* of the epithelial cells lining the duct, and those portions where the duct changes are all due to intra-epithelial spread of cancer.

It is possible that the intraductal growth arising from the lining epithelial cells *in situ* may assume a variety of forms or that all stages from a precancerous to a cancerous state may be present; or that it may have multicentric foci of origin in one or more ducts near their outlets; or that it may even dominate the picture so far as the ducts are concerned, the intra-epithelial spread in such cases being more extensive in epidermis than in ducts. In my experience, however, the initial ductal lesion arising from the lining epithelial cells *in situ* is usually inconspicuous and difficult to identify with certainty. This difficulty in identification of the primary growth may result from a close structural resemblance between the primary growth and its intra-epithelial cancerous extension; or the difficulty may be due to the primary growth being masked or obliterated by its intra-epithelial cancerous extension; or the primary growth may be small and situated to one side of the nipple so that it is not included in the sections cut for routine examination, which are usually taken from the middle of the nipple. Even if in Paget's disease upward intra-epithelial extension of cancer is seen in the nipple close to the epidermis, this would not invalidate the theory that intra-epithelial spread of cancer down one or more ducts forms a link between the skin lesion and the infiltrating cancer in the underlying breast, because the primary growth in a duct may be sufficiently deeply placed for its cancerous intra-



epithelial extension in an upward direction to be so early that it would be separated from the epidermis by a portion of unaffected duct.

I should like to mention here that Ewing<sup>2</sup> quoted me as having found the ducts intact in eight cases. This is incorrect. In my monograph I said: "On eight occasions after a careful examination was made of the whole breast no scirrhus cancer was detected, the disease being limited to ducts and epidermis without any evident extension of growth into the connective tissues." In point of fact I have never seen a case of Paget's disease of the nipple in which the ducts were free from cancer, and in a large percentage of the cases which I have examined, cancer had already infiltrated the connective tissue of the underlying breast, so that Paget's disease of the nipple should always be regarded as of serious significance.



Text-Fig. 2

The view held by Muir<sup>3</sup> as to the relation of the epidermal lesion to the infiltrating cancer in the underlying breast is "that the underlying and causative condition of both Paget's disease and the accompanying cancer is intraduct carcinoma, that is, a condition in which malignancy is present within ducts but has not broken through their walls. . . . It may occur in any part of the duct system and it may arise in multiple independent foci. If it occurs in the ducts of the nipple it may spread to its epidermis, Paget's disease resulting; this is a rare occurrence. The common event is a break-through in some part of the breast with carcinoma as the result." Under the heading "The relation of Paget's disease to carcinoma of the breast," Muir says: "In the first place, discontinuity between intraduct disease at a higher level and that at a lower can frequently be traced. The disease in the nipple may, in fact, be completely cut off from that in the breast."

Text-Figure 2 is a diagram showing in duct 1 two independent foci

of duct carcinoma arising from the lining epithelial cells *in situ*. One of these foci is in the nipple at L and the other in the underlying breast at M. From the focus in the nipple, cancer has infiltrated the epidermis, Paget's disease resulting. From the focus in the underlying breast, the duct cancer has broken through the wall and infiltrated the connective tissue with carcinoma of the breast as the result. The disease in the nipple is completely cut off from that in the breast. In Text-Figure 2 two completely independent primary cancers are represented, one infiltrating connective tissue, the other infiltrating epithelium, whereas in Text-Figure 1 all of the diseased parts are linked up with one primary cancer (duct 1,A).

In studying any disease it is desirable to eliminate complicating factors and to examine lesions in a pure state, and at as early a stage of their development as possible. In studying Paget's disease, it is therefore desirable to concentrate on those cases which are uncomplicated by infiltrating carcinoma of the breast, and Muir's group of five cases<sup>3</sup> in which "intraduct carcinoma was confined to the nipple" are of special interest. Suppose that these breasts where the nipple only was involved had not been amputated; then judging by experience in other cases, a cancer infiltrating the connective tissue would have formed in the underlying breast at a later date in each instance. I suggest that this infiltrating cancer in the breast would have resulted from downward extension.

In regard to the order of appearance of the epidermal lesion and the infiltrating carcinoma in the underlying breast Paget,<sup>4</sup> in his original paper "On Disease of the Mammary Areola Preceding Cancer of the Mammary Gland," wrote: "I believe it has not yet been published that certain chronic affections of the skin of the nipple and areola are very often succeeded by the formation of scirrhus cancer in the mammary gland. I have seen about fifteen cases in which this has happened, and the events were in all of them so similar that one description may suffice." No mention is made in this paper of mammary cancer preceding the lesion of the nipple.

In only 1 of the 24 cases included in my published series<sup>1</sup> was there clinical evidence suggesting that the epidermal lesion appeared after the cancer had formed in the underlying breast. If, in Paget's disease associated with infiltrating carcinoma of the underlying breast, there are two separate and independent infiltrating cancers as represented in Text-Figure 2, it is surprising that the cancer in the underlying breast should not more often precede the cancer in the nipple (with its cutaneous extension).

In considering the question of discontinuity between an affected

part of a duct in the nipple and the disease deep in the breast, it is important to recognize the distinction between ordinary duct carcinoma and the special duct carcinoma of Paget's disease. In ordinary duct carcinoma there may be discontinuity with multiple foci of origin and various stages of activity, but in the special duct carcinoma of Paget's disease there is no true discontinuity. The question of discontinuity of the special intra-epithelial cancer of Paget's disease, whether in epidermis or in ducts, may be considered from three points of view:

1. Apparent discontinuity is common near the advancing margin of the lesion whether in epidermis or in ducts, and this apparent discontinuity is probably due to death of some of the cancer cells.

2. In the epidermal lesion apparent discontinuity may be due to irregular rate of growth at the advancing margin of the lesion; in some areas at the periphery of the lesion advance may be rapid, in others slow. Sections cut in certain planes through these areas may show zones of epidermis invaded by cancer separated by zones of epidermis free from cancer. In the cramped space in ducts there is less scope for such an arrangement of alternating cancerous and noncancerous areas than there is in the epidermis, but even in the ducts this may occur to a slight extent near the advancing margin of the lesion.

3. In the main body of the epidermal lesion there may be areas in which the epidermis is completely destroyed by the intra-epithelial cancer, these areas being superficial ulcers with floors formed of granulation tissue. In affected ducts there may be areas where there is no epithelium on the wall, and where the lumen is occupied by necrotic cancer cells. The point which calls for special emphasis, however, is that in the main track of the ductal lesion there is never a considerable length of duct lined by normal epithelial cells.

The special duct cancer of Paget's disease may be present in the upper part of a duct and ordinary duct cancer be present in the lower part of the same duct, the two affected parts being separated by a considerable length of normal duct. I suggest that this is the explanation of the following statement made by Cheate and Cutler<sup>5</sup> in their chapter on Paget's disease of the nipple: "In describing other cases of this series ducts were also depicted in which neoplastic epithelial growth was limited to their upper parts and which, after long intervals of normal ducts, led into the epithelial neoplasia situated in their final distributions. In many of these terminal ducts were also papillomatous formations which may be assumed to be of primary spontaneous growth arising in the epithelium of those structures as the disease in the upper ducts was nonpapillomatous."

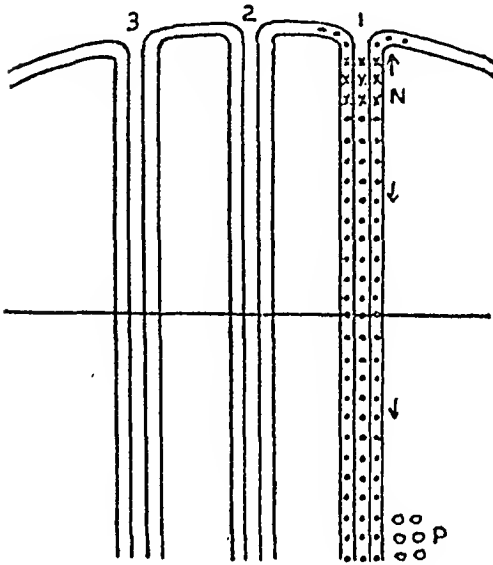
In some cases of Paget's disease the neoplastic overgrowth which constitutes the initial lesion may not be limited to a duct or ducts near their outlets, but may be present also in other separate foci in ducts in the underlying breast. Should this occur, the situation might arise where a focus of the initial neoplastic epithelial overgrowth in a duct near its outlet would have become cancerous and spread by the intra-epithelial route down that duct or (via the epidermis) down neighboring ducts. This spread down the ducts might have extended to different levels; in some it might have extended down to the acini (Text-Fig. 1, duct 3), in others it might have extended only a short distance down the ducts and so involved only their upper parts (Text-Fig. 1, duct 4). In the deeper parts of one or more of these ducts (Text-Fig. 1, duct 4,J), whose upper parts are the seat of full-fledged cancer spreading downwards by the intra-epithelial route, there might be separate foci of initial neoplastic epithelial overgrowth of the same order as that which became carcinomatous near a duct outlet (Text-Fig. 1, duct 1,A) and later infiltrated epidermis and ducts by intra-epithelial spread. This deep focus of initial neoplastic epithelial overgrowth (Text-Fig. 1, duct 4,J) might be at any stage of transition from a benign condition to duct carcinoma, and papillomatous formation might be one of the appearances seen early in the transition; a variety of stages in the transition might be present at any one time. In such a duct (Text-Fig. 1, duct 4) the upper part (D and E) would show the special duct cancer of Paget's disease, the lower part (J) would show ordinary duct cancer, and the two parts would be separated by a portion of the duct free from growth. Even if this discontinuity were present in some ducts, continuity of cancer spreading by the intra-epithelial route from the upper limits of ducts down to the acini might be present in other ducts (Text-Fig. 1, ducts 1, 2, and 3), so that infiltration of connective tissue deep in the breast might come from either ordinary duct cancer (Text-Fig. 1, duct 4,J) or from special duct cancer of Paget's disease (Text-Fig. 1, duct 1,G, or duct 2,H).

POSSIBLE SEQUENCE WHEN, AS OCCASIONALLY HAPPENS, PAGET'S  
DISEASE OF THE NIPPLE IS PRECEDED BY INFILTRATING CANCER IN  
THE UNDERLYING BREAST

Text-Figure 3 represents what I suggested in my monograph<sup>1</sup> as the most likely sequence of events. A focus of duct cancer which has arisen from the lining epithelial cells *in situ* near the duct outlet is represented in duct 1 at N. This cancer has spread down the duct by the intra-epithelial route and broken through the wall of the duct in the underlying breast at P with infiltrating cancer as the result. Only after this

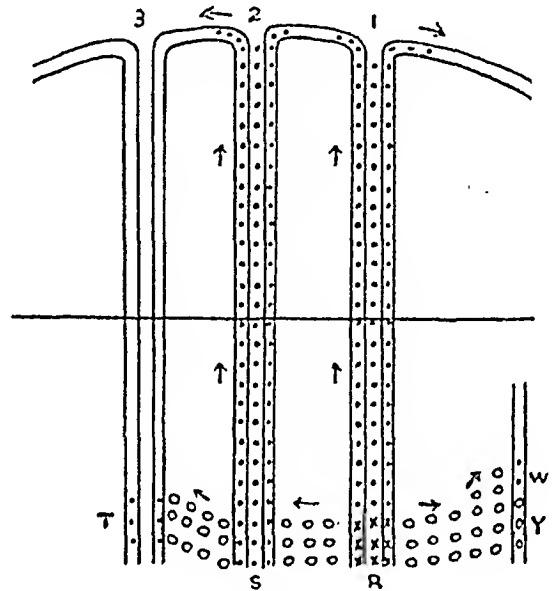
had occurred did the focus of duct cancer at N infiltrate the epidermis by the intra-epithelial route.

Another possible sequence of events is represented in Text-Figure 4. A focus of duct cancer which has arisen from the lining epithelial cells *in situ* in the underlying breast is represented in duct 1 at R. This cancer has broken through the duct wall and invaded the connective tissue of the breast. From the connective tissue the cancer has spread on the left into the deep part of duct 2 at S and duct 3 at T, and on



Text-Fig. 3

- xxx  
xxx Cancer arising from lining epithelial cells *in situ*
- ::: Cancer infiltrating epithelium
- ooo  
ooo Cancer infiltrating connective tissue



Text-Fig. 4

the right it has spread to involve the skin of the breast at W and Y. From the primary focus of duct cancer at R or from the secondarily invaded deep part of duct 2 at S cancer has spread upwards by the intra-epithelial route and entered the epidermis producing lesions essentially the same as that found in Paget's disease uncomplicated by infiltrating cancer in the underlying breast.

The reasons why the sequence of events represented in Text-Figure 3 was suggested in my monograph<sup>1</sup> as the most likely explanation when the infiltrating cancer in the underlying breast preceded the epidermal lesion are as follows:

1. In some cases of uncomplicated Paget's disease the epidermal lesion is very small and yet the ducts are extensively involved by cancer, both in the nipple and in the underlying breast, so much so that the appearances suggest that cancer might break through the duct

walls and infiltrate the connective tissue in the underlying breast at any moment. It is not going much further to suppose that this breakthrough might occur before there is infiltration of the epidermis by the primary duct cancer near the duct outlet.

2. No doubt in many infiltrating cancers of the underlying breast the cancer cells in the connective tissue irrupt into uninvolved ducts as in Text-Figure 4, duct 3,T, and intra-epithelial spread up the duct commences, but before this intra-epithelial cancer spreads far (as in Text-Fig. 4, duct 2), it is overwhelmed by the neighboring cancer spreading in the connective tissue. Similarly, when infiltrating cancer spreads through the connective tissue of the breast to involve the epidermis as at W in Text-Figure 4, cancer cells may at first enter the epidermis and produce appearances similar to those of the epidermal lesion in Paget's disease, but the rapid growth of the infiltrating cancer in the connective tissue destroys the skin and a malignant ulcer forms. Occasionally the cancer cells may spread a short distance in the squamous epithelium at the edge of the malignant ulcer, but they seldom spread far enough to produce a condition like that in the epidermis in Paget's disease. Dunn,<sup>6</sup> however, has described invasion of the cuticle by a mucinous carcinoma at the lower end of the external anal sphincter.

Ascending duct cancer in breasts where infiltrating cancer already exists has been described and illustrated by Muir,<sup>3</sup> but the evidence in his illustrations is, in my opinion, not altogether convincing; in particular his Figure 6 is, I think, open to another interpretation. The legend to this illustration is as follows: "Fig. 6. Intraduct carcinoma in duct of nipple, of older standing in lower part and just reaching the orifice. Case 2.  $\times 18$ ." Referring to his case 2 (from which his Figures 6 and 7 were taken), Muir said: "Extensive intraduct carcinoma was present in the nipple and breast, also infiltrating cancer in the breast and in the lower part of the nipple. One duct in the nipple was completely filled with Paget cells right up to the orifice. . . ." The duct in the middle of Muir's Figure 6, presumably the one which he said was completely filled with Paget cells right up to the orifice, contains no cells in its lumen in the lowest part in this illustration, and the duct wall in this situation appears free from growth. This appearance is against spread of cancer up the duct from a lower level. The small duct shown on the left of this figure is not referred to by Muir, but it appears to be the seat of cancer, especially in the upper part. As this duct is traced downwards it is seen to break up into what look like acini, some of which seem to be free from cancer. In this duct the cancer seems older in the upper part than in the lower part, the

appearances, in my opinion, being in favor of downward rather than of upward spread of cancer. These comments notwithstanding, I consider that when Paget's disease of the nipple is preceded by infiltrating cancer in the underlying breast, either of the sequences of events represented diagrammatically in Text-Figures 3 and 4 may have occurred.

## ILLUSTRATIVE CASES

### *Case 1*

The breast (P.U.S. 2332) was amputated because of a "sore" which had been present on the nipple for 6 months. There was no pain and no discharge. Immediately before operation the nipple was slightly redder than normal, and a scab was adherent to its summit. No other abnormality was detected, and the axillary lymph nodes were not enlarged. Simple amputation of the breast was carried out.

The nipple was bisected vertically, and serial sections were cut from each half. (The serial sections, however, did not include the entire nipple.) In addition, blocks of tissue were taken from the underlying breast, and in four of these cancer was present within ducts, or within ducts and acini, but no convincing evidence of cancerous invasion of the connective tissue of the breast was found.

The appearance of a vertical section cut through the middle of the nipple is to be seen in Figure 1. The area of skin involved is small; this area is easy to define because of the zone of inflammatory cells in the dermis. Just to the left of the center of the illustration a large duct, involved throughout its whole extent, presents a striking feature. To the right of this duct are three others all affected in their upper parts but otherwise free from growth. The transverse clefts about the level of the base of the nipple are artifacts. On the right-hand side, deep to the nipple, are some slightly dilated ducts free from growth. To the left of these, and at about the same level, is an ill-defined dilated duct affected by growth throughout. Still farther to the left, at this level, is a clear area (adipose tissue) in which a large blood vessel is conspicuous.

In Figure 2 the portion of Figure 1 enclosed within the rectangle is shown more highly magnified. The upper parts of several ducts are included. That on the left is extensively invaded above and free from growth below. That in the middle is free from growth except at the extreme upper limit which is involved by growth, though this cannot be appreciated in the photomicrograph. To the right is a duct which is involved by growth throughout the whole extent above the transverse strip of connective tissue (which includes some involuntary muscle). The duct below this strip of connective tissue (probably a continuation of that above the strip) shows very early involvement in its uppermost part, and abundant epithelial cells in the lumen. The small

section of a duct, which looks like an inverted V, in the uppermost part of the illustration has cancer cells among the lining epithelial cells, though this cannot be appreciated in the photomicrograph.

The changes in the affected epidermis and ducts in this and other vertical sections of the nipple near its midline are akin, and can be understood by studying Figures 3 to 10 inclusive. Let us examine the epidermal lesion at an early stage (Fig. 3). Here we see cancer cells infiltrating the epidermis in its deeper part; to the left a cancer cell is to be seen separated by an appreciable distance from others that are evident as the cluster of cancer cells on the right is approached. It is not necessary to assume that the cancer cells wander in the epidermis; it is likely that many of the cancer cells die early, and that the apparently normal squamous epithelium between the cancer cells has actually been traversed by the growth. Another possibility is that continuity between the cancer cell extensions would be found in other planes of section.

When a more advanced stage of the epidermal lesion is examined, changes of longer standing in the epidermis are met with. These vary considerably in appearance. In some parts (Fig. 4) the surface of the epidermis remains intact and invading cancer cells are arranged in masses separated by thin layers of epidermis. In other situations the individual cancer cells are separate, and as a rule are more numerous in the deeper part of the epidermis, though sometimes they are scattered throughout its substance. In still other situations the epidermis has been destroyed by the growth, the condition being one of superficial ulceration, vascular connective tissue forming the floor of the ulcer. In spite of minor differences in histological characters, the epidermal lesion is fundamentally the same throughout, and is due to intra-epidermal invasion by carcinoma.

When the lesion is examined in affected ducts near their outlets the appearances in the duct and in the epidermis are essentially the same. Figure 5 shows the upper limits of three ducts opening together on the free surface of the nipple. In this plane of section only one of the ducts can be seen extending deep into the nipple. The resemblance of the ductal lesion to the epidermal lesion is especially close near the outlet of the duct, because the lining is here composed of squamous epithelium. As the duct is traced downwards, appearances like those in Figure 10 are to be seen. There is, however, considerable variation in the compactness or looseness of arrangement of the cancer cells in the affected ducts.

The special form of duct carcinoma present in ducts affected by Paget's disease is best illustrated by Figures 6, 7, and 8. In Figure 6



the upper part of the duct is affected, the lower part is free from growth; the affected portion is involved continuously down to the advancing margin. Near the advancing margin the growth is seen to be spreading in an intra-epithelial fashion; the cancer is spreading down the duct not as a solid column but as a hollow cylinder. A similar appearance is evident near the advancing margin of cancer in the duct illustrated in Figure 8. The part of Figure 6 indicated by the letter A is shown more highly magnified in Figure 7 in which the lining epithelium is to be seen resting on connective tissue to the left. The lining epithelial cells, which have small, darkly stained nuclei, are separated into two thin layers: an intact layer on the free surface, and an irregular and incomplete deeper layer near the connective tissue. The separation is caused by a zone of cancer cells with large, rounded or oval nuclei staining more faintly than the nuclei of the lining epithelial cells. In many instances a darkly staining nucleolus can be seen near the center of the nucleus of a cancer cell. The cytoplasm of the cancer cells is fairly voluminous and stains faintly. The duct changes in Figure 7 are considered to be essentially the same as the epidermal changes in Figure 4.

Figure 8, which shows a different duct from that in Figure 6, illustrates the earliest stage of the special form of duct carcinoma of Paget's disease. The lowest limit of extension of the cancer down this particular duct is included in this figure. The duct wall at the bottom of the illustration is free from growth. The duct changes in Figure 8 are considered to be essentially the same as the epidermal changes in Figure 3. Two conspicuous isolated cancer cells are to be seen on the left of Figure 8 situated in the epithelial lining. Higher up the duct, on both sides, there are more numerous cancer cells in the epithelial lining. At the top of the illustration, the cancer cells are more abundant and have broken through the lining epithelium, which has been obliterated or mixed up with the cancer cells occupying the lumen in this situation. The cancer cells have not come from the epithelial cells lining this duct at this level, but are descended from the cells of the initial cancerous focus from which they have spread by the intra-epithelial route. Just above the lower of the two isolated cancer cells on the left of Figure 8 is an ill-defined, rather large cell which may be a degenerate cancer cell, and just below the upper of the two isolated cancer cells on the left of Figure 8 is another ill-defined cell which may be a degenerate cancer cell. The appearances are consistent with the opinion that the cancer traversed the epithelium between these two apparently isolated cancer cells but that some of the cancer cells have

died. Where the cancer insinuates its way down the duct by the intra-epithelial route, appearances like those shown in Figures 6 to 8 inclusive are met, but quite commonly the intra-epithelial spread down the duct is more rapid or destructive with the result that the presence of individual cancer cells in relatively intact lining epithelium is not to be seen.

Figure 9 shows appearances corresponding closely with those in Figure 8 except that below and to the right in Figure 9 there is a focus of cancer apparently isolated and separated by uninvolved lining epithelium from what looks like the advancing margin of the duct cancer on the left. This apparently isolated focus is regarded as part of the intra-epithelial spread. It is exceptional to find such a wide gap of uninvolved lining epithelium in duct cancer spreading by the intra-epithelial route. In this particular instance the uninvolved lining epithelium between the advancing cancer on the left and the focus of cancer on the right may have been traversed by the cancer, but sections in other planes seem to show a link between the apparently isolated focus on the right and the collection of cancer cells on the left which are just short of the advancing margin in this situation.

An important point that must be stressed is that any semblance of discontinuity in cancer extending by the intra-epithelial route is limited to the region where the cancer is actually spreading; once the cancer reaches the stage represented in the upper parts of Figures 6, 8, and 9 or throughout the whole of Figure 10, then normal lining epithelium is destroyed. It is true that the cancer cells in an affected duct may undergo necrosis and that no live cells may be present either in the lumen or on the wall of an involved duct at certain levels, but there will not be a return to an appearance resembling that of a normal duct.

The later stage of the ductal lesion is well shown in Figure 10, and this is the appearance which is usually such a striking feature of the ducts in Paget's disease of the nipple. The changes are essentially the same as those in the epidermal lesion (Fig. 4), but in the epidermis the squamous epithelium is firmer and not so easily disintegrated as is the epithelium which lines ducts.

In Figure 10 the duct is occupied by cancer cells which have no special arrangement. There may be a few of the original lining epithelial cells mixed up with the cancer cells, but in such older parts of the ductal lesion the cancer cells dominate the picture. Sometimes the lining epithelial cells are abundant in the lumen near the advancing margin of the ductal lesion and slightly beyond this advancing margin;

this will be referred to later (see Fig. 18). If the irregular arrangement of the cancer cells in the upper parts of the ducts illustrated in Figures 6 and 8 is compared with the appearances shown in Figure 10, it will be seen that there is a close resemblance between them. In this particular breast all of the affected ducts except A in Figure 12 show appearances which seem to be essentially the same as those illustrated in Figure 10; that is to say, they show these appearances where the ductal lesion is well established, but at the advancing margin the appearances are like those shown in Figures 6 and 8. In an occasional affected duct which resembles that illustrated in Figure 10 there is a suspicion of glandular arrangement, the cancer not being completely undifferentiated.

It is recognized that appearances like those illustrated in Figure 10 may result from cancer arising *in situ* from epithelial cells lining ducts, and a definite opinion to the contrary could not be given if attention were paid to this area alone, but the appearances in Figure 10 are essentially the same as those in the upper parts of Figures 6 and 8 and it is thought that the evidence is strong that in Figures 6 and 8 we are dealing with the special form of duct cancer characteristic of Paget's disease.

Figure 11 shows a duct leading into acini; this duct is in one of the blocks of tissue taken from the underlying breast. In Figure 11 duct and acini are involved in cancer throughout. Much of the growth in the duct is necrotic. The isolated groups of epithelial cells to the left of the illustration are thought to be acini invaded by cancer.

It is thought that when duct cancer spreading downwards by the intra-epithelial route as illustrated in Figures 6 and 8 continues its downward extension, duct changes like those shown in Figure 10 result; and that when the downward spread extends to the acini, changes like those shown in Figure 11 result. That is the interpretation placed on Figure 11 though it is recognized that, taken purely on the evidence revealed by Figure 11, the changes could be due to neoplastic overgrowth of lining cells *in situ*.

The epidermal and duct changes which have been described so far (Figs. 1 to 11) are those which I regard as characteristic of Paget's disease of the nipple. In all of these ducts, and in the epidermis, the lesion is one of full-fledged cancer; there is no recognizable starting point, and no precancerous change is to be seen. In many cases, for example case 2, these changes, or others essentially like them, are all that are to be found in an ordinary routine examination of a breast affected by Paget's disease.

The description up to this point (except that related to Fig. 11) applies to sections near the midline of this nipple. When serial sections farther and farther away from the midline are examined, a section is reached where, in addition to ducts showing the characteristic changes already described, there is a duct showing different changes—changes which suggest that possibly the original neoplastic lesion started in this particular duct. This duct showing special features (along with many other ducts showing the features already described) is to be seen in the section illustrated in Figure 12. In this illustration some ten ducts are present; serial sections show, however, that what appear to be separate ducts are in some instances different portions of the same duct. All of the ducts in this illustration show similar histological appearances except the one marked A. After studying the serial sections, the impression was gained that though duct A is somewhat irregular in its distribution, it has two or three main branches. Apparently isolated groups of cancer cells in this and other situations in the nipple, as well as in the underlying breast tissue, might suggest that there is infiltration of connective tissue, but when serial sections are examined, many of these apparently isolated groups of cancer cells are seen to link up with ducts and acini of which they represent outlying parts. No convincing evidence of infiltration of the connective tissue was found in this breast, either in the nipple or in underlying mammary substance.

In duct A of Figure 12 there is neoplastic tissue with a distinctly glandular structure. This is well shown in Figure 13 which is not taken from the same section as Figure 12 but from a section close to it in the series. When duct A is traced downwards in the serial sections, the glandular cancer is seen to extend into the upper ends of the branches of this duct for a short distance, and then to merge insensibly in undifferentiated cancer which extends downwards by the intra-epithelial route as in the other affected ducts in this breast. When duct A is traced upwards in the serial sections, the glandular structure of the growth is evident almost to the level of the epidermis, but in the epidermis the growth shows no evidence of glandular structure. (Occasionally in Paget's disease the cancer does present a tendency to glandular structure even in the epidermis.) The appearances of the neoplastic tissue in Figure 13 suggest origin from the epithelium *in situ*. Even though the arrangement is fairly regular, the appearances are thought to be consistent with the growth being cancerous.

A striking contrast with duct A (shown also in Fig. 13) is presented by the other ducts in Figure 12, all of which are alike except that

some are involved by growth throughout, whereas others (B, C, D, E, F) are affected only in their upper parts. The five ducts affected only in their upper parts are shown more highly magnified in Figures 14 to 18 inclusive which are all taken from the same section as Figure 12. The changes in all of the ducts in Figure 12 (except A which is shown also in Fig. 13) are essentially the same as those illustrated in Figures 6 and 8, and are consistent with duct cancer spreading downwards by the intra-epithelial route. In some of these ducts, and especially in that illustrated in Figure 18, the lumen of the duct contains abundant epithelial cells well below the lowest limit of extension of the cancer. In this situation large numbers of the cells in the lumen are nonneoplastic epithelial cells thrown off the wall as a result of the cancerous invasion, though no doubt cancer cells are mixed with the nonneoplastic cells. As the cancer spreads down the duct, the cells which occupy the lumen where the lesion is fully established are practically all cancer cells.

While recognizing that cancer may spread by the intra-epithelial route and yet present a glandular structure, still the contrast between the structure of the growth in the upper part of duct A (Figs. 12 and 13) and that of all the other affected ducts in the breast is so striking that the following suggestion is submitted as a possible explanation of the sequence of events in case 1.

#### Possible Sequence of Events in Case 1

If we assume that the opinions already expressed concerning the changes in the epidermis and in the ducts are correct, then we have the following information to guide us:

1. Intra-epidermal cancer spreading by the intra-epithelial route is present in the epidermis on the summit of the nipple.
2. A large number of ducts in the nipple are the seat of duct cancer.
3. All but one of these affected ducts contain undifferentiated cancer.
4. The ducts showing undifferentiated cancer are to be seen at all levels in the nipple and also in the underlying breast.
5. In the underlying breast the undifferentiated cancer in ducts extends into acini.
6. Where ducts containing undifferentiated cancer are seen in particular sections to be affected in one part and not in another, it is invariably the upper part that is affected and the lower part that is unaffected.
7. In such ducts where the upper part is affected and the lower part is unaffected, the zone where these two parts meet shows spread

of the cancer downwards in the epithelial lining towards the uninvolved lower part of the duct.

8. When the uninvolved lower parts of such ducts are traced downwards in serial sections they are seen to be free from growth.

9. When the involved upper parts of such ducts are traced upwards in serial sections they are seen to be continuously involved up to the epidermis.

10. When such ducts are traced into the epidermis the changes in the ducts are continuous with the changes in the epidermis.

11. The changes in these ducts appear to be essentially the same as the changes in the epidermis, both at the advancing margin of the lesion and in those parts of the lesion which are more advanced and of longer standing.

12. The one affected duct which does not contain undifferentiated cancer contains cancer with a glandular structure (duct A of Figs. 12 and 13). The glandular cancer is situated in duct A high in the nipple. When this glandular cancer is traced upwards in duct A its glandular character disappears, and in the epidermis the cancer is all undifferentiated. When traced downwards this glandular cancer extends into the commencement of the branches of duct A but then merges insensibly in undifferentiated cancer which extends farther down the branches.

With this information before us the following suggestion is made as to the sequence of events in case 1. The disease commenced in this breast in one duct (duct A of Figs. 12 and 13) situated not in the middle of the nipple but somewhat towards one side of the nipple. The cancer arose from the lining epithelial cells *in situ* and assumed a glandular structure; it involved this duct from almost the level of the epidermis down to and including the upper ends of its main branches. This length of duct is regarded as the site of origin of the primary cancer. When the primary cancer in duct A extended from its site of origin downwards in the branches of duct A and upwards and outwards in the epidermis, it lost its glandular structure and spread as an undifferentiated cancer by the intra-epithelial route, no longer arising from the lining epithelial cells *in situ*. From the epidermis the undifferentiated cancer spread by the intra-epithelial route down many other ducts. In these ducts the downward extension reached various levels and in some of them it spread into the mammary tissue deep to the nipple.

In relation to Text-Figure 1 it is thought that the ducts in Figure 2 correspond with Text-Figure 1, duct 4; Figure 5 with Text-Figure 1,

duct 3 or duct 4,D; Figures 6, 7, 8, and 9 with Text-Figure 1, duct 4,E; Figure 10 with Text-Figure 1, duct 3 or duct 4,D; Figure 11 with Text-Figure 1, duct 3; Figure 12, duct A (Fig. 13) with Text-Figure 1, duct 1,A; Figure 12, ducts B (Fig. 14), C (Fig. 15), D (Fig. 16), E (Fig. 17), and F (Fig. 18) with Text-Figure 1, duct 4; and the remaining ducts in Figure 12 with Text-Figure 1, duct 3 or duct 4,D. In case 1 no break through the duct wall as in Text-Figure 1, duct 1,G, and duct 2,H, had occurred.

In case 1, intra-epithelial spread down pilosebaceous follicles is not to be seen and very early intra-epithelial spread down ducts is not striking, but these are both conspicuous in case 2.

### *Case. 2*

The patient (P.U.S. 2677) was an unmarried woman, 56 years of age, who said that a small eroded area had appeared on the right nipple 4 months before the breast was amputated. The eroded area had gradually spread to involve the remainder of the nipple and the whole areola. The nipple had always been retracted. Radical removal of the right breast and axillary lymph nodes was performed.

After fixation of the specimen the ulcer in the nipple area measured 2.5 cm. in diameter. The nipple was depressed. On bisecting the breast through the center of the nipple the skin in the nipple area was seen to be increased in thickness (maximum thickness, 0.7 cm.). In the mammary substance there was no cystic condition and no nodule of growth; indeed, the mammary tissue appeared relatively normal, though when it was cut it felt rather tough.

Microscopical examination revealed cancer in the epidermis of the nipple, also in ducts and acini of both nipple and underlying mammary gland, but no infiltration of the connective tissue was to be seen and none of the five axillary lymph nodes examined showed invasion by cancer.

Figure 19 shows epidermis infiltrated by cancer cells, some of which are large and some degenerate; the spaces are due to clefts between the cancer cells and the epidermal cells. In Figure 20 a single large cancer cell is present; its isolation is probably due to other cancer cells having died and disappeared. Figure 21 shows cancer spreading by the intra-epithelial route from the epidermis down into a pilosebaceous follicle. The lowest limit of spread on the right side of the duct at A is shown under high magnification in Figure 22 (A in Fig. 21 corresponds with A in Fig. 22). In Figure 22 the cancer cells in the upper part are in fairly good condition, but those in the lower part are degenerate, some being represented only by small dark-staining fragments.

Figure 23 shows cancer in the epidermis extending down the wall of a duct. Figure 24 shows the continuation of the same duct. Figure 24 was not taken from the same section as Figure 23 but from one near it in the series. Taken in conjunction, Figures 23 and 24 show appearances consistent with the opinion that the cancer has spread continuously by the intra-epithelial route from the epidermis down to the lower part of the duct in Figure 24. Figure 25 shows appearances consistent with the opinion that cancer in the epidermis has spread by the intra-epithelial route into the uppermost part of an accessory duct. Figure 26 includes the commencement of a main lactiferous duct which is regarded as showing a later stage in secondary cancerous involvement due to cancer spreading by the intra-epithelial route from the epidermis down the duct, and later disrupting the epithelial lining of the duct and setting cancer cells free in the duct lumen. Changes like those illustrated in this figure are present in ducts deeper in the nipple and also in the underlying breast, but no appearances suggesting a primary focus were detected anywhere in the sections of nipple and underlying breast which were examined.

In relation to the diagrammatic representation in Text-Figure 1 it is thought that Figures 21 and 22 correspond with Text-Figure 1,F; Figures 23, 24, and 25, with Text-Figure 1, duct 4; and Figure 26 with Text-Figure 1, duct 3 or duct 4,D.

#### SUMMARY AND CONCLUSIONS

1. An account is given of changes that occur in Paget's disease of the nipple, with special reference to the changes in the ducts.

2. Two cases are reported in which these changes are illustrated.

3. A distinction is drawn between ordinary duct cancer in which the growth arises as a result of the proliferation of the lining epithelial cells *in situ* and the special duct cancer of Paget's disease in which the cancer cells have infiltrated from a distance as part of a centrifugal spread by the intra-epithelial route from the primary duct cancer which has arisen in a duct near its outlet.

4. In this special duct cancer of Paget's disease, the special quality is in the intra-epithelial mode of spread. It is not suggested that there is anything special about the cancer cells.

5. It is recognized that occasionally the nipple lesion and the mammary cancer may be two completely independent primary infiltrating cancers: that in the nipple, infiltrating epithelium; that in the underlying breast, infiltrating connective tissue.

6. Evidence is submitted, however, in support of the opinion that



usually in Paget's disease of the nipple the epidermal lesion precedes the infiltrating cancer in the underlying breast, and is linked to this infiltrating cancer by spreading down one or more ducts by the intra-epithelial route (special duct cancer of Paget's disease).

7. Points in this evidence are:

(a) Cases are met with at all three of the following stages: (1) involving only the epidermis of the nipple and ducts of the nipple; (2) in addition to (1), involving ducts and acini in the underlying breast; (3) in addition to (1) and (2), infiltrating the connective tissue in the underlying breast.

(b) In all of Paget's 15 cases and in all but 1 of the 24 cases in my published series,<sup>1</sup> the skin lesion preceded the cancer in the mammary tissue (as judged by clinical standards).

(c) Cases of Paget's disease uncomplicated by infiltrating carcinoma in the underlying breast provide microscopical evidence of intra-epithelial downward spread of cancer (special duct cancer of Paget's disease) to different levels in ducts and in pilosebaceous follicles.

(d) In some of the ducts where the evidence in support of downward spread of cancer is strong, the ducts look ripe for rupture which would result in cancerous infiltration of the connective tissue.

8. When, as occasionally happens, the infiltrating cancer in the underlying breast precedes the epidermal lesion, it is considered that there are two possible explanations:

(a) That the epidermal lesion is due to delayed intra-epithelial extension by a primary duct cancer arising from lining epithelial cells near the duct outlet, cancer having previously spread by the intra-epithelial route from the primary duct cancer down the duct into the underlying breast and broken through the duct wall in this situation with resultant infiltration of the connective tissue.

(b) That the epidermal lesion is due to irruption of cancer, infiltrating the connective tissue in the underlying breast, into a duct in this situation, with subsequent intra-epithelial spread up the duct into the epidermis.

9. In breasts amputated for Paget's disease of the nipple the skin lesion is always associated with duct cancer in the nipple. In the great majority of cases it is also associated with duct cancer in the underlying breast, and in a large percentage of cases cancer has already infiltrated the connective tissue of the underlying breast. Paget's disease, therefore, is always to be regarded as of serious significance.

I am indebted to the late Dr. Allan Gillies for providing me with the specimen from case 1 and to Dr. B. T. Edye for the specimen from case 2. I wish to thank Mr. L. Findlayson for technical assistance, Miss M. G. MacKenzie for secretarial assistance, and Mr. S. Woodward-Smith for drawing the diagrams and taking the photomicrographs.

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[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

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### PLATE I

FIG. 1 (case 1). The cut surface of the nipple after bisection.  $\times 4$ .

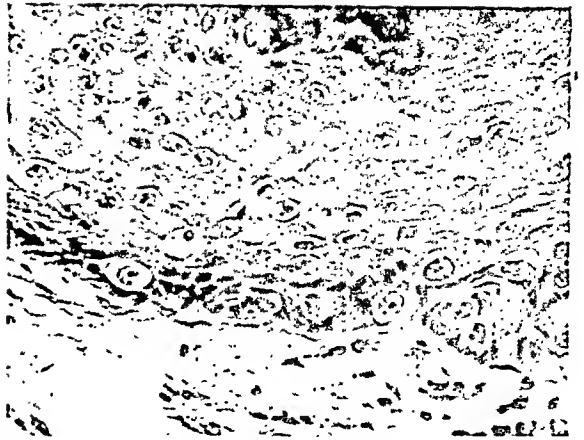
FIG. 2 (case 1). The portion of Figure 1 included in the rectangle is here shown more highly magnified.  $\times 30$ .

FIG. 3 (case 1). The epidermal lesion at an early stage.  $\times 275$ .

FIG. 4 (case 1). The epidermal lesion at a later stage.  $\times 250$ .



1



3



2



4

PLATE 2

FIG. 5 (case 1). Cancerous ducts joining the epidermis.  $\times 60$ .

FIG. 6 (case 1). Special duct cancer spreading downwards.  $\times 50$ .

FIG. 7 (case 1). The portion of Figure 6 indicated by A is here shown more highly magnified.  $\times 450$ .

FIG. 8 (case 1). The advancing margin of special duct carcinoma spreading downwards.  $\times 275$ .

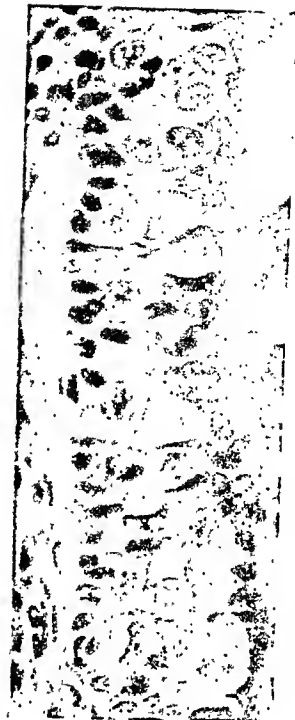
FIG. 9 (case 1). Special duct carcinoma showing apparent discontinuity at advancing margin.  $\times 200$ .



5



6



7



8



9

Inglis

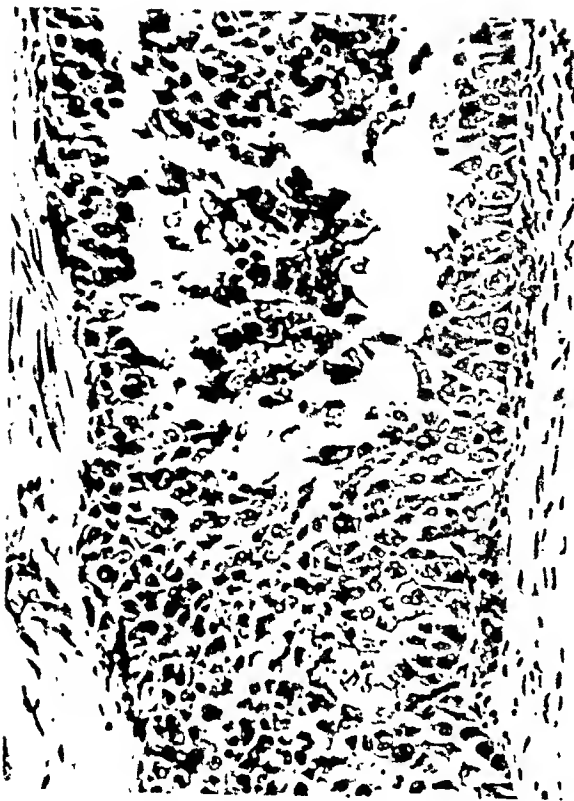
Paget's Disease of the Nipple

PLATE 3

FIG. 10 (case 1). Special duct carcinoma at an advanced stage. The appearances are also consistent with ordinary duct carcinoma.  $\times 180$ .

FIG. 11 (case 1). Special duct carcinoma with involvement of acini. The appearances are also consistent with ordinary duct carcinoma.  $\times 100$ .

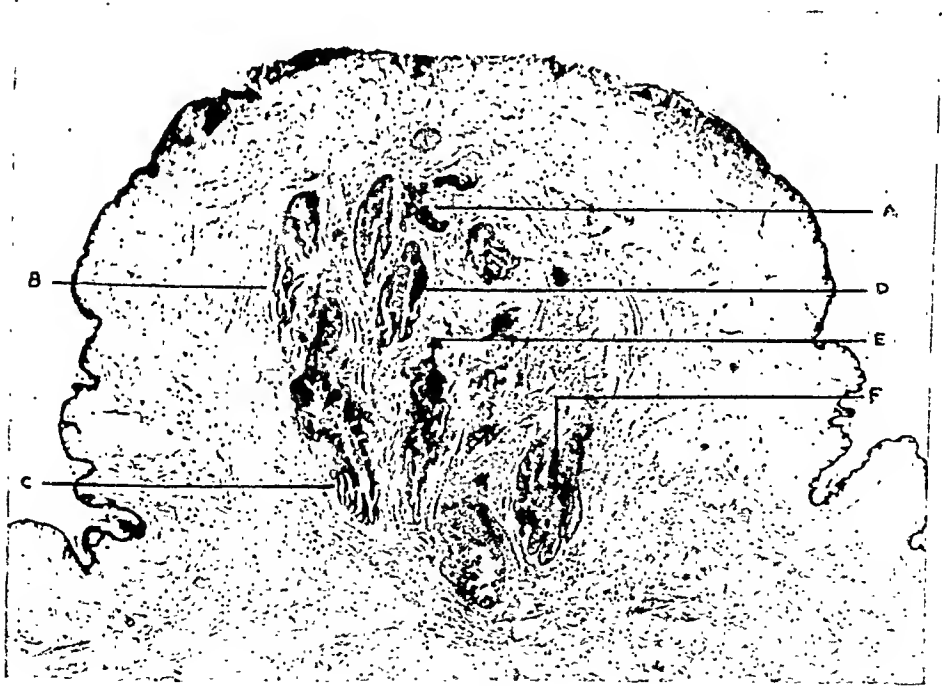
FIG. 12 (case 1). A vertical section through the nipple some distance from the midline.  $\times 9$ .



10



11



12

Inglis

Paget's Disease of the Nipple



PLATE 4

FIG. 13 (case 1). Portion of duct A in Figure 12 is here shown more highly magnified. (This photomicrograph is not taken from the same section as Figure 12 but from a section near it in the series.)  $\times 160$ .

FIG. 14 (case 1). Duct B of Figure 12 is here shown more highly magnified.  $\times 80$ .

FIG. 15 (case 1). Duct C of Figure 12 is here shown more highly magnified.  $\times 25$ .

FIG. 16 (case 1). Duct D of Figure 12 is here shown more highly magnified.  $\times 55$ .

FIG. 17 (case 1). Duct E of Figure 12 is here shown more highly magnified.  $\times 35$ .

FIG. 18 (case 1). Duct F of Figure 12 is here shown more highly magnified.  $\times 45$ .

(Figs. 14 to 18 inclusive were all taken from the same section as Fig. 12.)



13



14



15



16



17



18

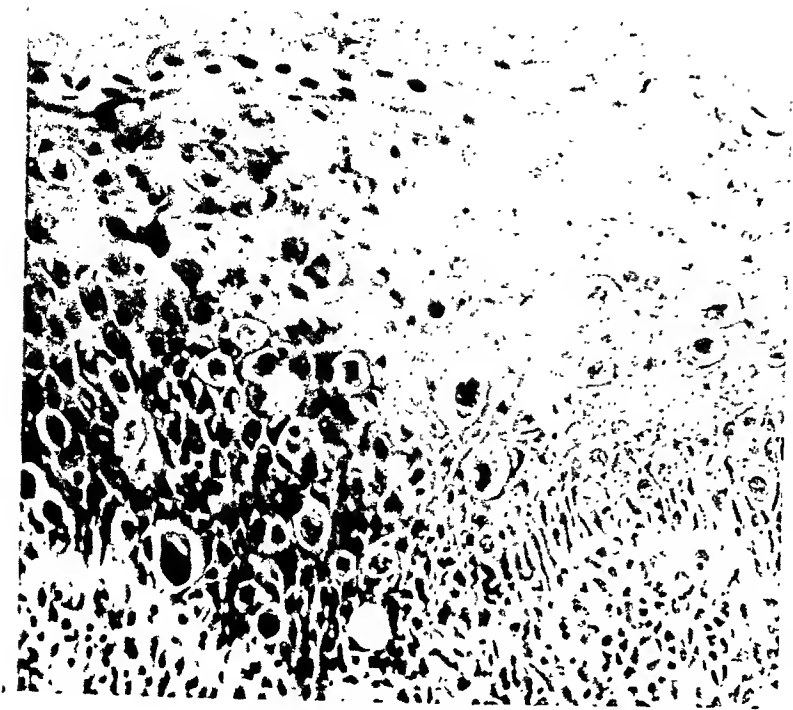
PLATE 5

FIG. 19 (case 2). The epidermal lesion.  $\times 200$ .

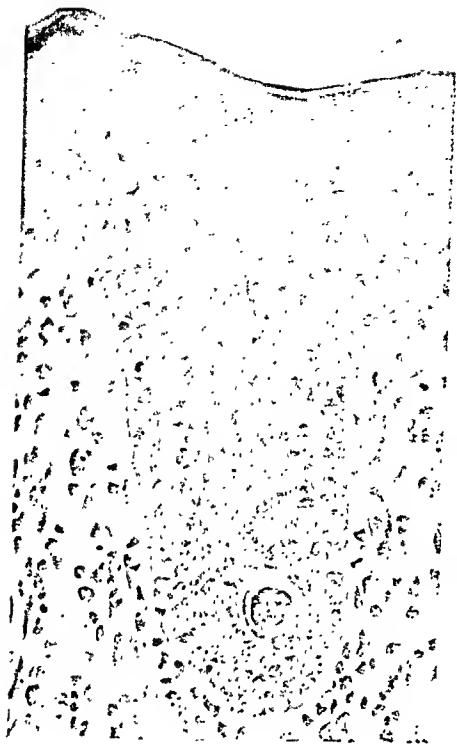
FIG. 20 (case 2). Isolated cancer cell in interpapillary process.  $\times 200$ .

FIG. 21 (case 2). Cancer spreading down wall of pilosebaceous follicle.  $\times 50$ .

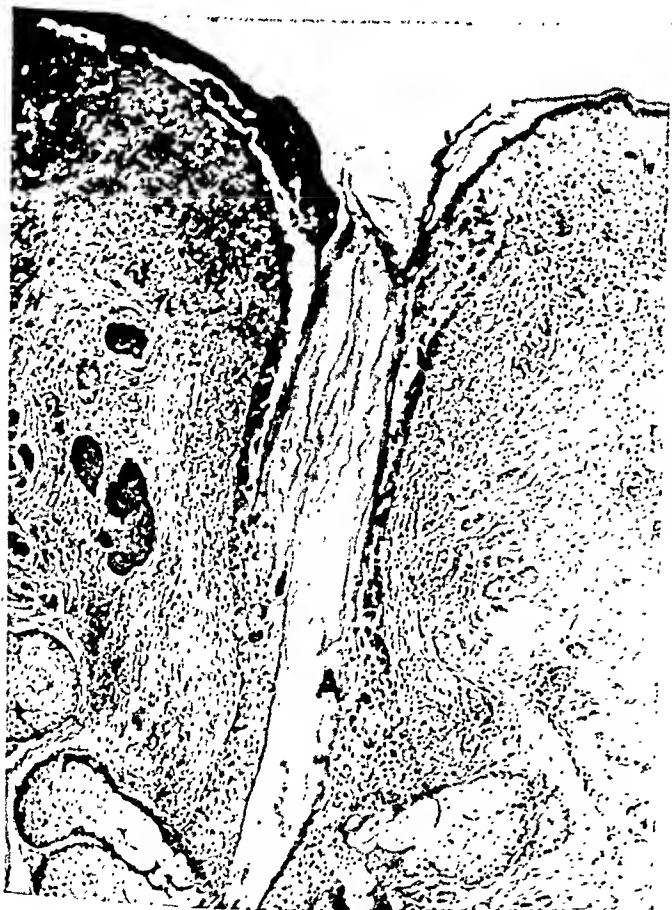
FIG. 22 (case 2). Portion of Figure 21 under higher magnification. A in Figure 21 corresponds with A in Figure 22.  $\times 200$ .



19



20



21



22

Inglis

Paget's Disease of the Nipple

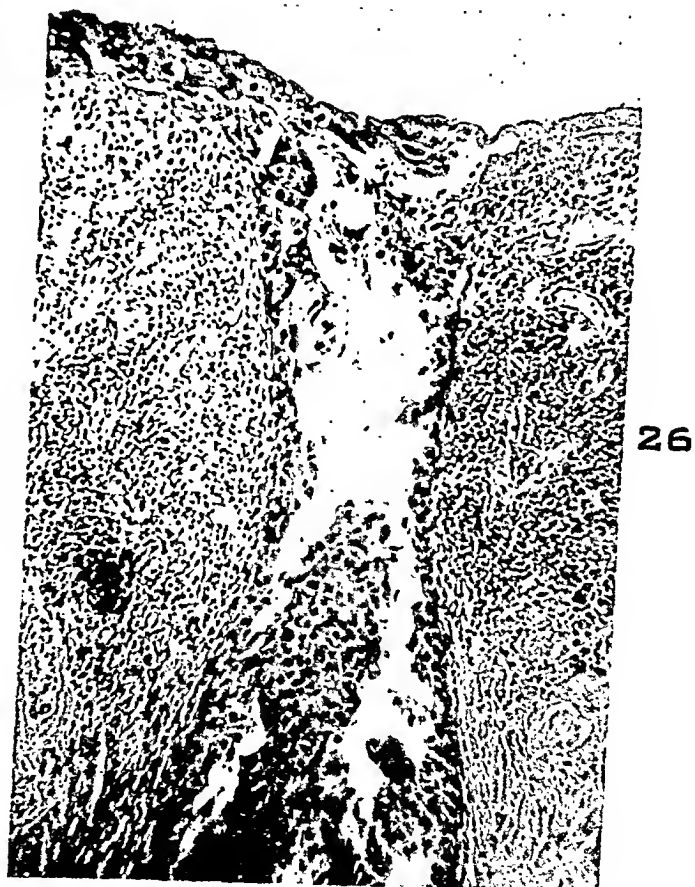
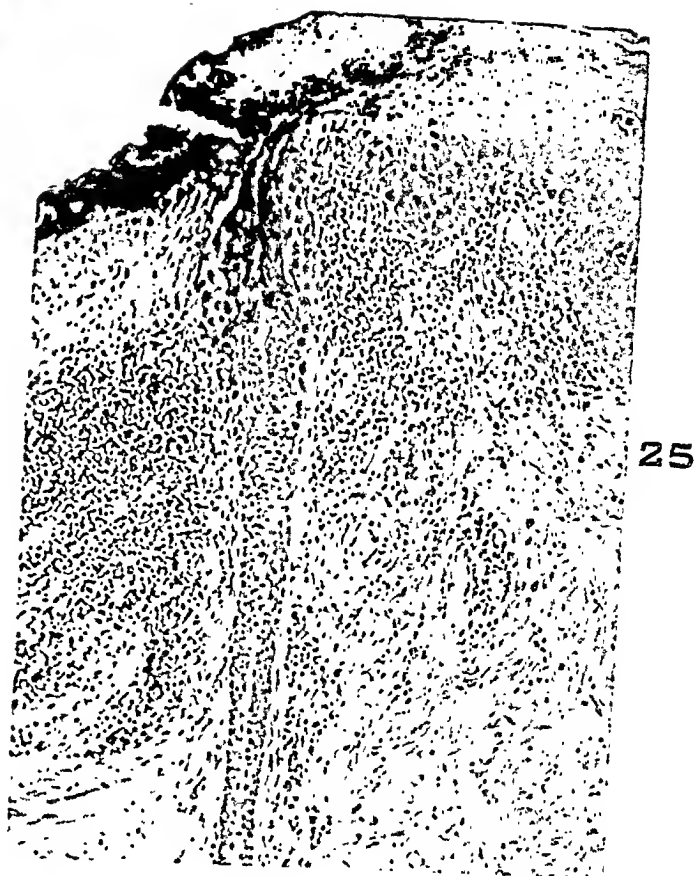
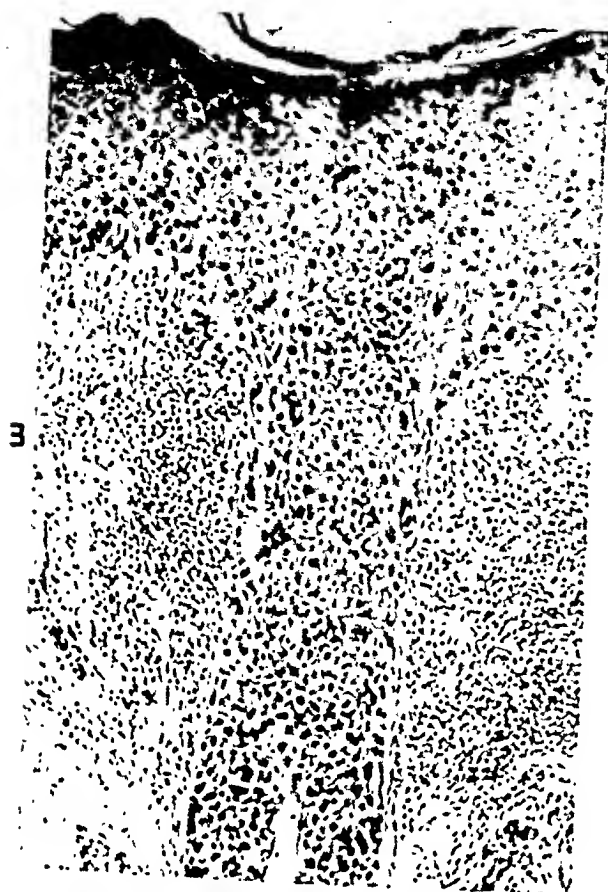
## PLATE 6

Figure 23 (case 2) and Figure 24 (case 2) are photomicrographs of the same duct.

Figure 24 is not taken from the same section as Figure 23 but from one near it in the series. The lower limit of Figure 23 corresponds with the upper limit of Figure 24. Taken in conjunction, these two figures show the whole length of the intra-epithelial spread of cancer down the duct from the epidermis. In Figure 23 the cancer is continuous in the epidermis and in the duct. The lowest part of the duct in Figure 24 has not yet been reached by the downward-spreading cancer. Figure 23,  $\times 100$ ; Figure 24,  $\times 100$ .

Figure 25 (case 2) shows intra-epidermal cancer which has spread a short distance down an accessory duct by the intra-epithelial route.  $\times 100$ .

Figure 26 (case 2). A main lactiferous duct and adjacent portion of epidermis both showing extensive invasion by cancer.  $\times 100$ .



Inglis

Paget's Disease of the Nipple



# A CHRONIC GRANULOMATOUS DISEASE OF SWINE WITH STRIKING RESEMBLANCE TO HODGKIN'S DISEASE \*

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It is apparent to anyone who reviews the many reports which relate to the nature and etiology of Hodgkin's disease that two serious obstacles block the way to a satisfactory solution of the problems involved. One is that it has not been possible to reproduce Hodgkin's disease in any of the lower animals, and the other is that thus far no one has observed a natural entity in any of the lower animals that critical workers will accept as identical with Hodgkin's disease.

There are several publications which deal with disorders in lower animals that bear certain superficial resemblances to Hodgkin's disease. Only a few of these need be cited since, in each, the difficulty encountered (readily appreciated by both author and reader) is essentially the same: all observations have been made on animals about which little or nothing was known previous to post-mortem examination, with the result that these studies could be made only on the basis of pathologic anatomy. A disease, or diseases, in the lower animals producing changes bearing some resemblance to the lesions of Hodgkin's disease has been described in dogs by MacMahon<sup>1</sup> and by Stalker, Schlotthauer, and Feldman;<sup>2</sup> in rabbits by Medlar and Sasano;<sup>3</sup> in swine by Hodgson;<sup>4</sup> and in the horse by Runnells and Benbrook.<sup>5</sup> Through the courtesy of several of these investigators we have had opportunities to study the materials forming the basis of their reports. In certain ways the diseases described present likenesses to Hodgkin's disease, but in none of the reported cases does the general picture more than suggest it. Even in those cases where the resemblance is most striking, the unavoidable incompleteness of the gross and histologic observations leaves the reader unconvinced.

The report which follows is subject to some of the criticism just directed toward earlier studies in this field; however, the cases are sufficiently numerous and the opportunity for gross and histologic study of the reactions involved has been comprehensive enough to make possible the recognition of a disease entity in the hog which if not actually Hodgkin's disease has a most impressive resemblance to it. To the best of our knowledge, this entity has not previously been described.

During the past 5 years one of us has been engaged in the study

\* Received for publication, April 5, 1945.



of what had appeared to be a close relationship between infection by brucella and Hodgkin's disease. This investigation necessitated an extended study of experimental brucellosis in a variety of animals, including swine.<sup>6</sup> In the course of this work when it was necessary to make collateral studies on natural brucellosis in this animal, the Federal Bureau of Animal Industry supplied most of the materials. One of us, while at the Pathological Laboratory of the United States Bureau of Animal Industry in Denver, Colorado, had collected materials from swine, some showing characteristic lesions of natural brucellosis and others, lesions suggestive of brucella infection. These materials came from a variety of sources; most of them were sent to the laboratory by veterinarians in the Federal Meat Inspection Service for investigation and diagnosis.

Whenever the circumstances permitted, the materials were studied both anatomically and bacteriologically. Preliminary studies showed that the material could be divided into two rather sharply defined groups: one composed of cases of brucellosis, in which the diagnosis had been established both by the anatomic and histologic character of the lesions and by bacteriologic and serologic methods; the other presenting lesions that were quite different from those found in the cases of brucellosis, none of which yielded organisms of any sort when cultured. Only one case belonging to the second group showed a significant agglutination titer for brucella. While there were these highly important differences between the cases of the two groups, the gross alterations of the tissues, particularly the lesions in the spleen, were sufficiently alike to suggest a possible relationship. In the routine histopathologic studies of the materials of the second group, a resemblance of the lesions to those of Hodgkin's disease was noted, and the cases were classified as "Hodgkin's-like" granuloma. In view of the fact that these lesions also bore a certain resemblance to the lesions in hogs, either suspected or known to be infected by brucella, it was considered possible that they might represent certain phases in the development of the lesions produced by brucella in the hog. In our concurrent studies of experimental and natural brucellosis, we have been able to show that brucellosis in the hog is unrelated to the Hodgkin's-like granulomatous process,<sup>6</sup> but we were unable to determine the cause of the granulomatous lesion. Its resemblance to Hodgkin's disease was found to be so striking in further studies of this group that we felt that the cases should be recorded. The number of cases and the diversity of materials available for study make possible the recognition of a definite pathologic entity. Since these cases may prove useful in future comparative studies, the case studied most comprehensively and significant variations in other cases of this series will be fully described.

## REPORT OF CASES

*Case 1*

Hog ABF 2, an almost fully grown hog, suspected of having leukemia because of pronounced enlargement of the superficial lymph nodes, was sent for complete autopsy to Dr. S. S. Blackman of the Johns Hopkins Department of Pathology who was studying experimental leukemia. At autopsy a general enlargement of the lymph nodes due to peculiar fibrous changes was found. This was accompanied by considerable swelling and a profound, presumably granulomatous alteration of the structure of the spleen. Since microscopic examination showed no evidence of leukemia, all of the histologic preparations were given to us to be used in our studies of the Hodgkin's-brucellosis problem.

Many sections from each of the organs in this animal were studied microscopically by a variety of staining technics. With the exception of the presence of Sarcosporidia in the fibers of the voluntary muscles and of the heart, the lesions in this case were confined to the lymph nodes, the spleen, the bone marrow, and the liver. A description of each of these organs follows.

*Lymph Nodes.* The normal architecture persisted in some of the nodes, but in others it was greatly altered. In practically all nodes small areas showed relatively normal structural relationships. The earliest phase of the development of the lesion was characterized by a pronounced infiltration of the peripheral sinuses by cells of many types. In this infiltrate there were recognized a great number of eosinophilic leukocytes, a still greater number of reticulo-endothelial cells, many of which clearly were phagocytes, relatively few small round cells resembling lymphocytes and plasma cells, and a good many unidentifiable mononuclear wanderers. This reaction was followed by the development of a pronounced reticulo-endothelial proliferation which in some places converted the lymph node into a dense connective-tissue-like reticular scar. In this scar large mononuclear cells in considerable variety and eosinophilic polymorphonuclear leukocytes were numerous. Areas of focal necrosis, sometimes accompanied by hemorrhage, were conspicuous. Giant cells with multiple nuclei, seen here and there, usually took the form of large multinucleated macrophage-like structures, but occasionally resembled Langhans' giant cells. There was nothing in any of the lymph nodes that resembled a genuine epithelioid reaction nor a tubercle. Giant cells of the Dorothy Reed type were not present. The lesion, thus, was distinctly granulomatous; it resembled rather strikingly the very dense sclerosing lesion that characterizes the far advanced stage of Hodgkin's disease. Virtually all of the lymph

nodes studied histologically showed this type of advanced sclerosing reaction (Figs. 1 and 30).

*Spleen.* In the spleen the lesions occurred regularly and most characteristically in relation to the malpighian bodies. The normal cells of these structures gradually gave place to cells of a totally different kind, which bore a striking resemblance to the mononuclear cells that characterize the Hodgkin's lesion. As this proliferative reaction developed, a great quantity of hyaline reticulum was laid down. Eventually the cellular components of the lesion disappeared, leaving the malpighian body converted into a hyaline scar. Grossly, this reaction appeared as tiny gray nodules throughout the splenic pulp. Another characteristic lesion, somewhat different in its development but similar in its outcome, consisted of marked proliferation of the reticulo-endothelium, forming relatively large nodules of a highly cellular character. The proliferating cells maintained a degree of morphologic uniformity which in places suggested genuine neoplasia, but the nodules thus produced, in time were converted into a dense reticular scar similar to that described in the malpighian body. Although the reticulo-endothelial hyperplastic reaction is important, equal emphasis should be placed upon the presence of great numbers of eosinophilic leukocytes, hemorrhage, and giant cells of several varieties. As the lesion developed it sometimes obstructed the arterioles to produce relatively large infarct-like areas. The coalescence of these lesions produced an extensive nodular induration of the greatly enlarged spleen. A quantity of iron-containing pigment was scattered throughout the much altered splenic substance. The general transformation of the spleen by this granulomatous process was indistinguishable from that of Hodgkin's disease of the advanced sclerosing type; it bore no resemblance whatever to other lesions in the human spleen with which we are familiar. The cell complex that made up this granulomatous reaction was that of Hodgkin's disease, and the different types of giant cells that accompanied the mononuclear and polynucleated cells can be duplicated in sections from lesions of comparable age and development in the Hodgkin's process. Were this lesion found in the human spleen, the case would be readily accepted as one of Hodgkin's disease (Figs. 3, 13, 14, 15, and 16).

*Bone Marrow.* The bone marrow from one of the long bones and from the body of one of the vertebrae was studied. In both locations the lesions were focal. The cytologic composition of the lesion in the bone marrow was in every way comparable to that in the spleen. The lesion was distinctly a granuloma, characterized by a mixture of mononuclear cells, chiefly plasma cells and what appeared to be lymphocytes, leukocytes, and giant cells filling the meshes of a hyaline reticu-

lar scar. Some of the lesions were older than others, the tissues being almost nothing but scar. The uninvolved bone marrow had a normal cellular composition (Figs. 6, 28, and 29).

*Liver.* There was a diffuse round cell infiltration throughout the portal fibrous tissue of the liver. In some places there was considerable extension of this infiltration into the neighboring lobules, resulting in destruction of many of the liver cells. In affected areas a rich reticulum developed, thereby increasing the thickness and the density of the fibrous tissue capsule that normally surrounds the hepatic lobules. In addition to these periportal granulomatous infiltrations a similar reaction sometimes occurred within the lobule, presumably independent of the portal reaction. These intralobular lesions gave rise to extensive destruction of the hepatic parenchyma and its replacement by a granulomatous reaction. The cells which made up the reaction within the lobule and also in the portal areas were predominantly small, round elements, mixed with many eosinophilic leukocytes and a variety of mononuclear cells not easy to identify. It was obvious from the character and distribution of the lesions that long-continued progress of the disease followed by healing of the lesions eventually would result in an extensive cirrhosis of the liver. The obvious pathogenesis of the actively progressing but early lesions in this case makes it easy to understand the regular occurrence of cirrhosis of the liver in the other cases of this series. In the hepatic granulomatous reaction there was little production of giant cells. In all other respects the hepatic lesion was essentially the same as that in the spleen, the lymph nodes, and the bone marrow (Figs. 7, 8, 9, and 11).

In none of the lesions was it possible to demonstrate organisms of any variety either by culture of the tissues or by special staining of the histologic preparations.

In view of the clinical suspicion that this animal was suffering from leukemia, it should be emphasized that studies of blood clots in sections revealed no evidence of an alteration in the cytologic composition of the circulating blood. In the sections, furthermore, nothing was found to indicate a leukemic process.

### *Case 2*

Hog ABF 9, D-344, was approximately 1 year old. At post-mortem examination, Dr. H. L. Shorten of the Federal Meat Inspection Service, Denver, Colorado, noted multiple tumor-like nodules, approximately 5 to 8 mm. in diameter, in the spleen, the liver, and the kidneys. The liver showed pronounced cirrhosis. The nodules in the spleen and the liver were white and fibrous; those in the kidney were similar but perhaps somewhat softer. No data relating to the condition of the

other organs were available. The lymph nodes were not mentioned in the very brief protocol. No material was available for studies of agglutination titers or for bacteriologic examination. Our observations of the small pieces of tissue from spleen, liver, and kidneys added nothing to the original descriptions made by Dr. Shorten, but further information was gained from histologic studies.

*Spleen.* The lesions in the spleen in this case were identical with those in case 1, which appeared to have developed first in the malpighian bodies. The youngest lesions were characterized chiefly by proliferation of large mononuclear cells. The subsequent development of this reaction was featured by a certain amount of necrosis and marked production of hyaline reticulum; the end-result was the transformation of the malpighian body into a rather dense reticular scar. Accompanying the proliferation of the large reticulo-endothelial cells was an accumulation of eosinophilic leukocytes and a variety of small round cells. Hemorrhage was conspicuous. The larger nodules seen grossly in the spleen were the result of proliferation of the reticulo-endothelial cells accompanied by the formation of many multinucleated giant cells. The development of the reticular ground substance in these nodules was impressive. As in case 1, the lesions appeared much like tumors. Nodules of this type eventually lost their cellular character and in the end consisted of rather dense reticular scars in which a few eosinophilic leukocytes, multinucleated giant cells, and a variety of small mononuclear cells persisted. The unaltered splenic substance lying between the lesions showed considerable congestion, probably from portal obstruction, the result of accompanying cirrhosis of the liver (Figs. 4, 12, 17, and 20). (See also Figs. 18 and 19.)

*Liver.* The liver had dense periportal scarring throughout, which divided it into many small compartments, each containing a little mass of liver cells which belonged to the original lobule. The great bands of scar tissue that surrounded these lobules contained the bile ducts and showed a marked inflammatory reaction of a granulomatous character. The composition of the exudate here was like that in the splenic lesions. In the active granulomatous reaction, giant cells were inconspicuous just as they were in case 1. The more outstanding features were the great number of eosinophils and the extensive production of scar tissue (Fig. 10). (See also Figs. 2, 7, and 11.)

*Kidney.* Small nodular lesions, exactly like those in the spleen in so far as cellular composition is concerned, were present in the kidney. Giant cells, however, were not conspicuous; the eosinophilic reaction, on the other hand, was conspicuous.

In none of the sections were there bacteria or parasites of any sort.

The lesions in the spleen were identical with those in case 1, and if seen in the human spleen would, without question, be diagnosed Hodgkin's disease of advanced type. The lesions in the liver were like those in case 1, with the exception that the process had advanced much further, resulting in greater destruction of the hepatic tissue and more pronounced periportal scarring.

### Case 3

Hog ABF 10, D-548, was 1 year old. Autopsy was performed by Dr. I. L. Barstow of the Federal Meat Inspection Service, Denver, Colorado. The liver was diffusely scarred, enlarged, and indurated. Similar changes were noted in the kidneys, and tubercle-like foci were scattered throughout the spleen. No other gross abnormalities were noted. The gross appearance of the spleen, as we saw it, was not unlike that in cases 1 and 2, with the exception that the lesions in this case seemed to be much older because of their indurated, fibrotic character. The many small nodules scattered irregularly throughout the splenic tissue were usually discrete and well circumscribed, but not definitely encapsulated. Here and there they tended to coalesce. In gross appearance the lesions were like those of tuberculosis or brucellosis, and our first impression was that they were due to brucella infection. A considerable degree of what appeared to be scarring was obvious in the tissue from the kidneys. The appearance of the liver was that of diffuse nodular cirrhosis. In the more fibrous areas between the nodules of hepatic cells little yellow foci were found, suggesting either necrosis of liver cells or a granulomatous inflammatory reaction. A description of the histologic appearances of the spleen, liver, and kidney follows.

*Spleen.* Scattered throughout the sections of the spleen were small, rather sharply defined nodules which consisted chiefly of scar tissue containing clumps of eosinophilic leukocytes. The outer margin of each of the nodules showed extensive infiltration by eosinophilic leukocytes and small mononuclear wandering cells. The lesion was not encapsulated by fibrous tissue, but condensation of the normal splenic tissue toward its outer margin clearly defined its limits. There were no giant cells. Here and there were spindle-shaped spaces like those sometimes seen when crystals are dissolved from the tissues. There was a considerable amount of iron-containing pigment in the lesion. There was virtually no lymphoid tissue present in the spleen. The malpighian bodies were almost devoid of cells, which had been replaced by scar tissue.

It is evident that the typical splenic lesion in this case differs con-

siderably from that found in cases 1 and 2. Our interpretation of the lesions in this case is that they represent a granulomatous process in an advanced stage of healing. Although the lesions are now thought to represent the complete healing of a process identical with that seen in cases 1 and 2, they were considered originally to be the completely healed lesions of brucellosis, like those we have described in a previous communication<sup>6</sup> (Figs. 18 and 19).

*Liver.* The sections from the liver showed extensive periportal scarring. In the thick, dense bands of fibrous tissue surrounding the little nodules of persistent hepatic tissue there were collections of eosinophilic leukocytes and a variety of wandering cells. The lesion, like that in case 2 (Fig. 10), is interpreted as a granulomatous periportal hepatitis which has undergone almost complete healing.

*Kidney.* No active lesions were found in the kidney, but there was extensive interstitial scarring; in some areas this was focal, in others diffuse. Nothing was seen to indicate the nature of the process responsible for destruction in the kidney.

#### Case 4

The post-mortem examination of hog ABF 38, D-675, was also performed by Dr. I. L. Barstow. He observed that the spleen was enlarged to several times its normal size, with suet-like foci distributed throughout. The liver also was enlarged and was thought to show some evidence of degeneration. The submaxillary, portal, and renal lymph nodes appeared hyperplastic. Material consisting of lymph nodes, spleen, liver, and bone marrow was made available to us for study, in which we were able to confirm Dr. Barstow's observations. The lymph nodes we found to be firm in consistency, mottled, and rusty brown. They were not unlike those seen in advanced Hodgkin's disease. The lobular structure of the liver was prominent, suggesting definite cirrhosis. The spleen was remarkable in appearance with scattered little gray foci, elastic in consistency, which were rather sharply defined but not encapsulated. They varied greatly in size, some being just barely visible, others reaching a diameter of from 2 to 3 mm. In some places the lesions coalesced and produced a complete distortion of the splenic structure. Although there was some necrosis, the greater alteration of the tissue appeared to be the result of replacement of the normal splenic substance by the proliferation of a new kind of tissue. Pigmentation by iron was so pronounced that in some places the spleen had a definite porphyry-like appearance. The alterations in the splenic substance were indistinguishable from those regularly observed in

Hodgkin's disease. The gross lesions in the lymph nodes, liver, and spleen are shown in Figure 2. A histologic description of the lesions follows.

*Lymph Nodes.* The general architecture of the lymph nodes did not appear significantly disturbed, but there were areas in which a granulomatous inflammatory reaction caused considerable change. These areas were most numerous at the periphery of the node and along the course of the lymphatic sinusoids that lead toward the medulla of the gland. The reaction was similar to that in case 1, except that, as yet, little reticulo-endothelial scarring had occurred, indicating that the reaction in the lymph node was of a much earlier type. Another feature whereby it differed was the presence of many giant cells which were predominantly of two varieties. One was a multinucleated structure that was in every way like the Dorothy Reed cell of Hodgkin's disease. The other was a large, usually multinucleated but sometimes mononucleated cell with an abundance of cytoplasm which was dense and which stained rather intensely with eosin. In some instances the single nucleus in this giant cell was drawn out into a long cylindrical mass, and sometimes it appeared as a single central mass with several lobulations of irregular outline. Giant cells of the latter type were like those seen sometimes in the splenic pulp and always in the bone marrow. These megakaryocyte-like giant cells appeared in abundance also in the spleen. Another feature in the lymph nodes which was more conspicuous in this case than in case 1 was the presence of relatively large foci of proliferating mononuclear reticulo-endothelial cells. Eosinophils and other mononuclear cells were found, but not in sufficient numbers to suggest a neoplastic reaction. These little foci were like those that appeared in the spleens of cases 1 and 2. (They also formed a conspicuous proportion of the lesions in the spleen in this case.) Altogether, the lesion in the lymph node was a granuloma which, if seen in a human lymph node, would surely have been considered entirely compatible with human Hodgkin's disease (Figs. 31 and 33).

*Spleen.* The reactions in the spleen in this case were identical with those in case 2, being most conspicuous in connection with the malpighian bodies. The cytologic composition of the lesion was that of a granuloma. Eosinophils were prominent, and large mononuclear cells of varied structure, scattered among the fibrils of a rather dense reticulum, were characteristic. Among the mononuclear cells were many giant cells, some of them like the Dorothy Reed cells of Hodgkin's disease, others like the megakaryocytes of the bone marrow, and still others like large multinucleated fibroblasts. A striking feature of



the nucleus of the multinucleated giant cell was the sharply defined, slightly eosinophilic nucleolus. Similar nucleoli were commonly found in the mononuclear cells of the large variety which represented a cytologic type basically the same as that of many of the giant cells. Between the lesions, which were somewhat focal in distribution, the splenic pulp was sparse, the sinusoids were packed with blood, and there was some increase in fibrous tissue. Intense pigmentation of the scarred areas by deposits of iron was characteristic. The lesions, which obviously developed as small foci, tended to coalesce, leaving a little intervening, normal, splenic substance. Some of the sharply outlined nodules were composed of proliferating reticulo-endothelial cells with a pronounced reticulum; they resembled in every respect the lesions in the lymph nodes and suggested a genuine neoplastic development. An interesting and important feature of the small tumor-like areas of reticulo-endothelial proliferation was the presence of multinucleated giant cells like those already noted in other lesions of the spleen. It is clear that the alteration of the splenic substance in this case conforms to the histologic pattern which is characteristic of the lesions in all cases included in this report. The reaction here, however, appears more active than in any of the other cases except, perhaps, case 2. The reaction in the spleen is indistinguishable from that of Hodgkin's disease (Figs. 22 to 27).

*Liver.* The sections of the liver showed a considerable periportal granulomatous inflammation in which eosinophilic leukocytes were prominent; the other cells were chiefly small mononuclear elements. There were no giant cells, and very little reticulo-endothelial scarring. The periportal markings of the liver lobules were conspicuous, but there was not sufficient alteration of the hepatic structure to justify one in describing this liver as cirrhotic, although in the gross this organ did seem to be distinctly nodular.

*Bone Marrow.* Nothing unusual was seen in the bone marrow which was composed entirely of fat.

The blood serum of the animal from which these tissues came was positive for brucella agglutinins in a dilution of 1:50. A diphtheroid organism was recovered in culture from the spleen. Guinea-pig inoculations of the tissues produced no disease.

### Case 5

The materials available for our study of hog ABF 52, 14,300, were limited to small sections of spleen, liver, and one portal lymph node. Leukemia was suspected by Dr. G. A. Franz of Indianapolis, who did the autopsy, and the animal was sent to the Denver laboratory for diagnosis. The liver was found to be greatly enlarged, extensively

scarred, and finely nodular. The gross picture of the greatly enlarged spleen was identical with that of case 4. The lymph nodes from the hilum of the liver were enlarged and contained scattered, gray, poorly defined areas which tended to extend from the capsule toward the medulla. All tissues of this animal were slightly jaundiced. No bacteriologic studies were made. The changes in these tissues as seen microscopically may be described as follows.

*Spleen.* The lesion in the spleen conformed exactly to the pattern described in detail for case 4.

*Lymph Node.* The lesion in the lymph node was like that seen in the nodes of case 4 with the important exception that giant cells were more numerous and tended to conform to the pattern of the Dorothy Reed cell. Another point of difference is worth noting. The rather pronounced reticulo-endothelial reaction was pseudo-epithelioid in character in this node and, therefore, the lesion was composed of tissue of a somewhat homogeneous type. This was most noticeable at the periphery of the node where the lesion involved the peripheral sinuses, but it extended also along the lymphatic channels toward the medulla. Eosinophils were numerous. The architecture of the node was not greatly altered except in those areas where the lesions were best developed (Fig. 32).

*Liver.* There was extensive periportal scarring in the liver, but a receding granulomatous reaction was still active. Eosinophils were numerous. Mononuclear cells of considerable variety made up the rest of the exudate. Evident healing of the reaction had given rise to thick bands of fibrous tissue which surrounded masses of cells that represented original lobules. The histologic picture was that of extreme portal (biliary) cirrhosis.

### Case 6

Hog ABF 55, D-910, was 18 months old. At the autopsy, performed by Dr. O. E. Jung, Sr., of the Federal Meat Inspection Service, St. Louis, Missouri, the spleen was found to be greatly enlarged, perhaps eight times normal size. It was hemorrhagic, with numerous, small, suet-like foci distributed throughout. The liver was somewhat enlarged and showed a pronounced degree of cirrhosis. There was also enlargement of the lymph nodes. Neither bacteriologic nor serologic studies were possible nor was the material suitable for animal inoculations. Histologic sections of the materials available revealed the following changes.

*Spleen.* There was extensive scarring of the spleen, clearly the result of the coalescence of multiple lesions. These lesions appeared active only here and there but, wherever they were found to be progressing,

they were identical with the focal lesions described in cases 2 and 4. Since the general histologic picture conformed exactly to the pattern described in detail in these cases, further description of the lesions is unnecessary. It is obvious that the changes in the spleen are the result of a granulomatous process in an advanced stage of healing (Fig. 5).

*Liver.* Sections showed extensive periportal scarring of the liver, apparently the result of a granulomatous reaction which still could be seen here and there in the scar tissue. Many liver cells, particularly at the periphery of the lobules, had been destroyed. Although the process was still active, it was obvious that it was rapidly regressing.

*Lymph Node.* No histologic preparations of the lymphoid tissues were available.

### Case 7

The post-mortem examination of hog ABF 56, D-625, was performed by Dr. A. J. Clark, of the Federal Meat Inspection Service, Fort Worth, Texas. He found the spleen to be approximately 30 inches in length and correspondingly enlarged in width and thickness. The liver was enlarged and cirrhotic. The visceral lymph nodes were swollen and soft. There were no gross alterations of kidneys and lungs. The microscopic appearances of the tissues may be noted briefly.

*Liver.* There was a pronounced cirrhosis of the liver, the dense scarring being situated chiefly about the bile ducts in the portal areas. There was only slight activity of the destructive process, which appeared granulomatous as in other cases in this series.

*Spleen.* The picture in the spleen conformed in general to the pattern of the splenic alterations in the other cases of this group except that the original focal scarring had now become diffuse as a result of coalescence of the lesions. There was still some activity of the granulomatous process that preceded the scarring. This case is, in general, like case 5, in which the granulomatous inflammation had almost come to an end.

*Lymph Node.* The architecture of the node was not generally altered. There were foci of granulomatous reaction here and there along the course of the lymphatics extending from the capsule toward the medulla. The cytologic composition of this reaction was like that described for cases 4 and 5.

### COMMENT

When the pathologic anatomical data are examined critically, it becomes obvious that these cases present a clearly defined entity. Case 1 demonstrates that all of the tissues constituting the reticulo-endothelial

system are involved in the basic pathologic process. A consideration of the seven cases in the series leads us to the conclusion that one basic pathologic process, a granulomatous inflammation, is responsible for the lesions in every case. Focal and sometimes tumor-like proliferation of the reticulo-endothelial cells in some of the lesions in both spleen and lymph nodes need not lead to confusion since such pseudo-neoplastic processes are commonly found in many granulomatous reactions of proved causation.

The limitations placed upon the study of these cases by the circumstances under which the materials were obtained have made it impossible for us to obtain positive information about the cause. The lesions characteristic of these cases, when seen by veterinarians conducting meat inspection, are usually considered to be either granulomatous inflammation or neoplasia. Of our seven cases, three (cases 4, 6, and 7) were diagnosed clinically as lymphocytoma; two (cases 1 and 5), as leukemia; one (case 2), as sarcoma; and one (case 3), as tuberculosis. From our studies of natural and experimental brucellosis in the hog, it is possible to say that there is no indication that brucella has an etiologic relation to this granuloma nor is there any justification for considering any of the animal parasites or any form of *Mycobacterium tuberculosis* as a possible etiologic agent. In none of the lesions were we able to demonstrate organisms of any sort even though this series of seven cases represents all stages in the development of the granulomatous reaction which characterizes the entity. A careful study of the genesis of the lesions of all types in these cases leaves little support for the suggestion that they are neoplastic.

The almost constant occurrence of cirrhosis of the liver in these cases is worthy of comment. Primary destruction of the hepatic parenchyma followed by extensive scar formation and portal venous obstruction might easily give rise to enlargement and extensive scarring of the spleen. The type of reaction seen in the portal areas of the liver in most of this group might possibly follow chronic progressive hepatic destruction by a variety of agents. Thus it would be unnecessary to assume, as we have, that the hepatic granulomatous reaction and the lesions of the spleen are of identical, primary, specific causation; it is conceivable that the splenic lesion is simply a consequence of the hepatic portal scarring. However, even in this short series of cases, cirrhosis of the liver of such severity as to result in portal obstruction and scarring of the spleen was not always present. In fact, fibrosis and granulomatous reactions in the spleen (also in the lymph nodes) appear in some cases in which there is almost complete absence of

hepatic alteration; furthermore, the splenic lesion clearly is not a simple fibrosis like that which results from chronic congestion. In view of these facts, it is impossible to interpret these cases as instances of primary cirrhosis of the liver with peculiar sequellae. Even were one inclined to do this, the demonstration of generalized reticulo-endothelial disease in case 1, which was comprehensively studied, would make such a position untenable.

The entity recognized in this group of cases bears an extraordinary resemblance to Hodgkin's disease, if it actually is not that disease. We have attempted to make the comparison as objective as possible by supplementing the descriptive details in the protocols with illustrations.

In conclusion, it should be noted that a careful comparison of the lesions found in this group of cases with a variety of tumors in the hog, particularly the genuine tumors of the reticulo-endothelial system, as well as the leukemias, has shown conclusively that the cases do not represent variants of these diseases. This distinction has been made without difficulty. The observations made by one of us in the routine inspection of swine on the killing floors of packing houses as well as in the laboratory indicate that the entity described in this paper, like Hodgkin's disease, is relatively rare.

### SUMMARY

1. Seven cases of a relatively rare chronic granulomatous disease of swine are described. This disease of unknown causation, apparently not hitherto recognized as an entity, is characterized by a striking pathologico-anatomic resemblance to Hodgkin's disease, if it does not actually represent that disease in the hog. Further studies of this disease designed to determine its cause are in progress.

2. The unlikelihood that this Hodgkin's-like granulomatous disease is related to tuberculosis, brucellosis, or to any of the other granulomatous disorders of known cause is emphasized.

We wish to acknowledge our indebtedness to Dr. S. S. Blackman of the Department of Pathology, Johns Hopkins Medical School; and to Dr. H. L. Shorten and Dr. I. L. Barstow of Denver, Colorado; Dr. A. J. Clark of Fort Worth, Texas; and Dr. G. A. Franz of Indianapolis, Indiana, all of the Federal Meat Inspection Service, through whose careful observations the materials forming the basis of this study were accumulated. The senior author is particularly indebted to Dr. Adolph Eichhorn, formerly Chief, Pathologic Division, United States Bureau of Animal Industry, and to Dr. John R. Mohler, formerly Chief, United States Bureau of Animal Industry, whose cooperation has made possible not only this but other correlated studies.

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[ Illustrations follow ]

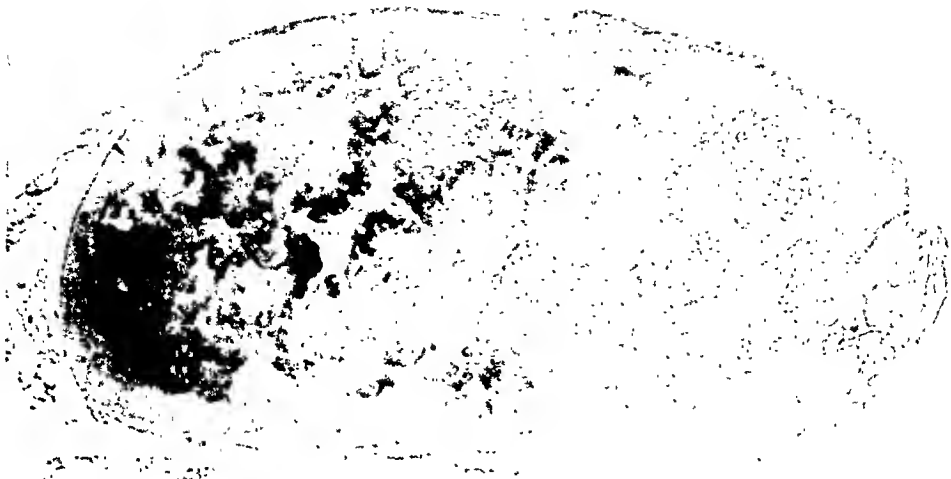
## DESCRIPTION OF PLATES

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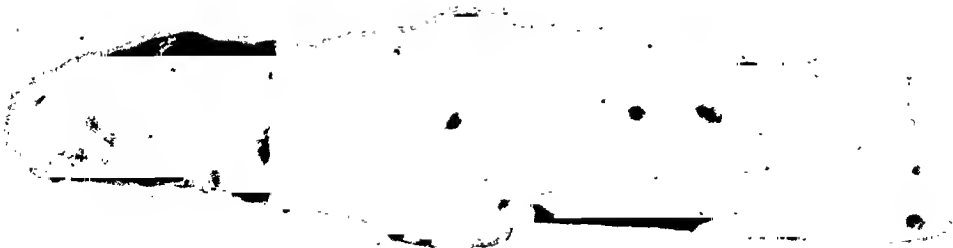
### PLATE 7

FIG. 1. Case 1. An enlarged and extensively scarred cervical lymph node in full section ( $\times 4$ ). The structure of the node is extensively altered by a granulomatous reaction resulting in a dense reticular fibrosis. Near the center and at the right, irregular areas of normal lymphoid tissue persist.

FIG. 2. Case 4. In the upper half of this photograph are three lymph nodes (one unsectioned, two sectioned), approximately actual size, which show enlargement and disturbed architecture. Below these are two blocks of liver, nodular in character, and at the bottom four blocks of spleen containing gray, granulomatous lesions.



1



2



### PLATE 8

FIG. 3. Case 1. A section of the spleen ( $\times 5$ ) shows great alterations in structure produced by a widely distributed granulomatous reaction. The very small white dots are completely scarred malpighian bodies. The minute tumor-like focal reticulo-endothelial proliferations are similar to the large tumor-like nodules shown in Figure 4 from another case in this series. (See Figs. 13, 14, 15, and 16 for higher magnifications.)

FIG. 4. Case 2. A section of the spleen ( $\times 5$ ) shows focal tumor-like reticulo-endothelial proliferation and accompanying diffuse and focal granulomatous alteration of the splenic pulp. (See Figs. 12 and 17 respectively for higher magnification of the lesion in the malpighian body and the granulomatous reaction in the pulp.) At higher magnification the large tumor-like nodules present a picture identical with that of the small gray foci in case 1 (Figs. 3 and 16).

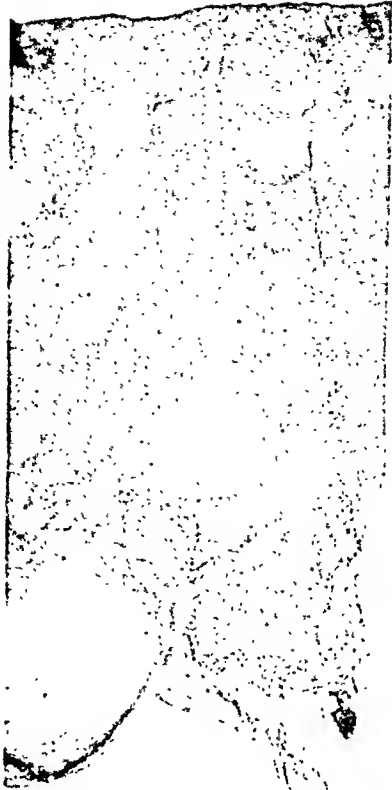
FIG. 5. Case 6. A section of the spleen ( $\times 5$ ) shows characteristic focal but coalescing granulomatous lesions. Figures 3, 4, and 5 together show the characteristic features of the spleen in this Hodgkin's-like granulomatous disease of swine.

FIG. 6. Case 1. A section of the bone marrow ( $\times 5$ ) shows granulomatous alteration and resulting scar. The lesion, essentially like those of the spleen and lymph nodes, is shown at higher magnification in Figures 28 and 29.

FIG. 7. Case 1. A section of the liver ( $\times 5$ ) shows a very early stage in the development of the periportal granulomatous inflammation. Higher magnifications are pictured in Figures 8, 9, and 11. In Figures 2 and 10 respectively are illustrated the gross and microscopic appearances of the far advanced and healing stages of this lesion in other cases of this series.



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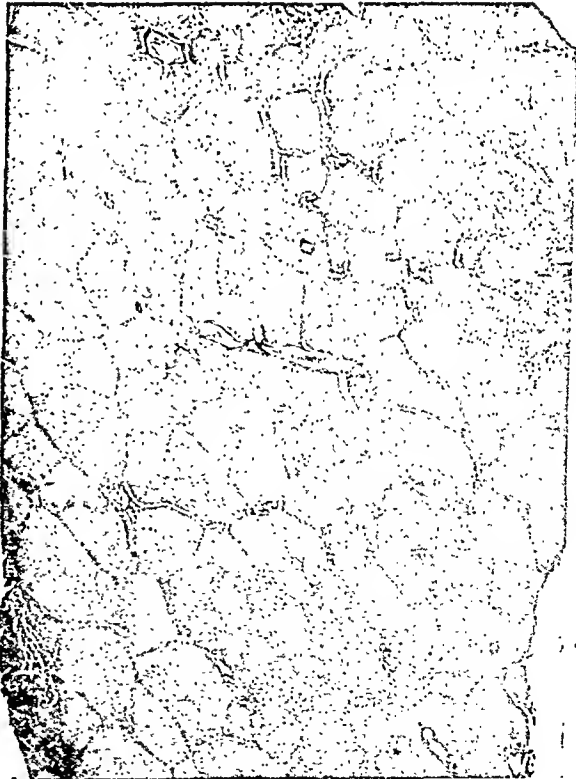
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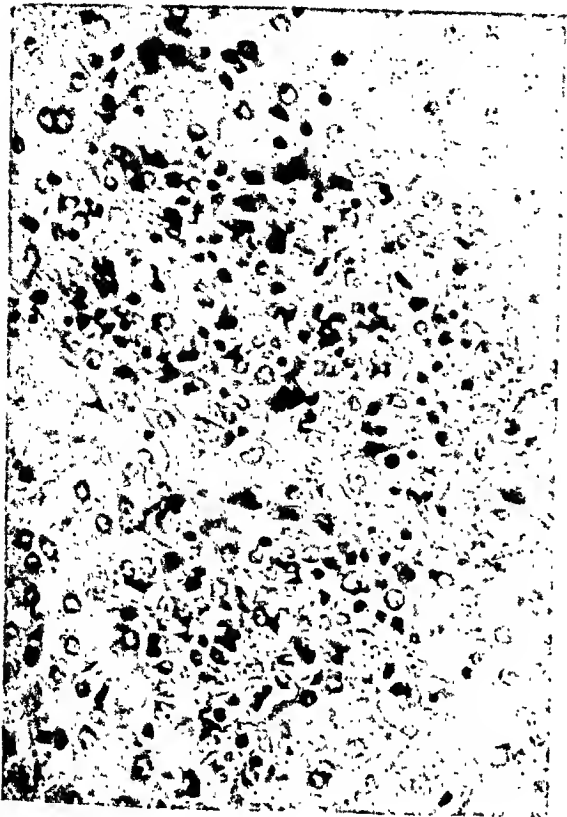


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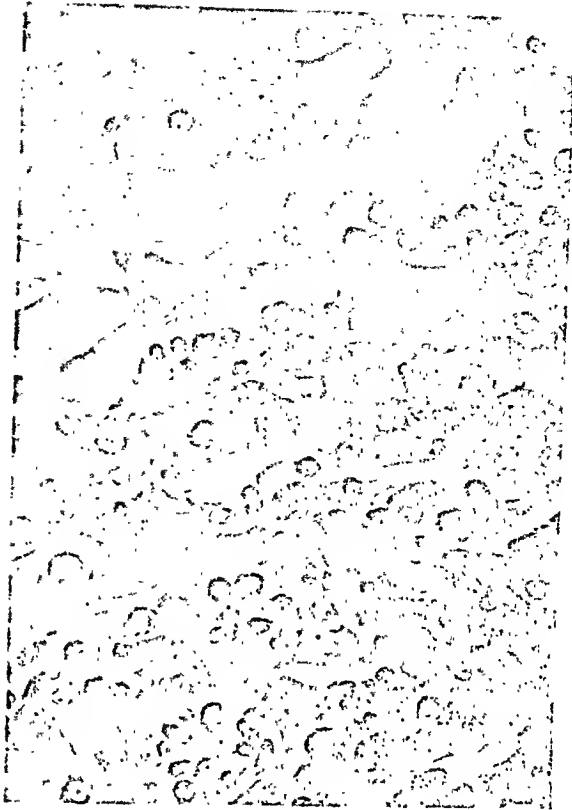
## PLATE 9

FIGS. 8, 9, and 11. Case 1. Photomicrographs showing the typical early and progressing granulomatous reactions in the liver characteristic of the Hodgkin's-like disease of the hog. Figure 8 shows the lesion within the hepatic lobule ( $\times 315$ ). Figure 9 is the same at a higher magnification ( $\times 485$ ). Figure 11 is a typical periportal lesion ( $\times 137$ ). (See Fig. 7 for a low-power view of this liver, and Figs. 2 and 10 for the advanced stage in the development of the hepatic lesion as found in other cases of this series.)

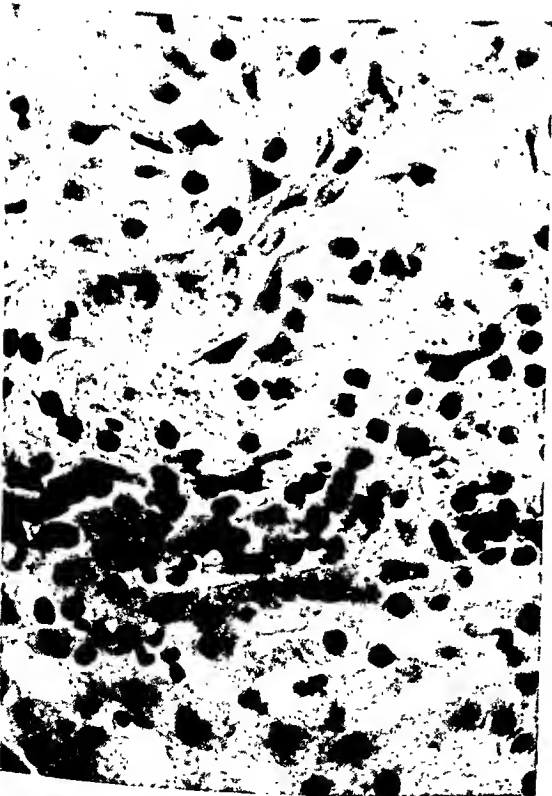
FIG. 10. Case 2. A section of the liver ( $\times 485$ ) showing the advanced healing stage of the periportal granulomatous inflammation characteristic of the Hodgkin's-like entity. Of note are the dense scarring and the peripherally situated, persistent eosinophilic and mononuclear reaction. For comparison with Figures 8, 9, and 11. The gross appearance of this liver was that of typical nodular cirrhosis (Fig. 2).



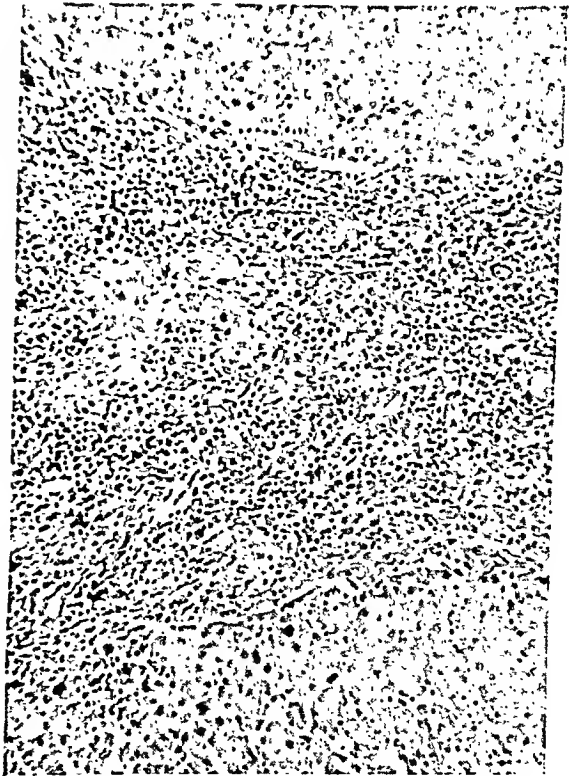
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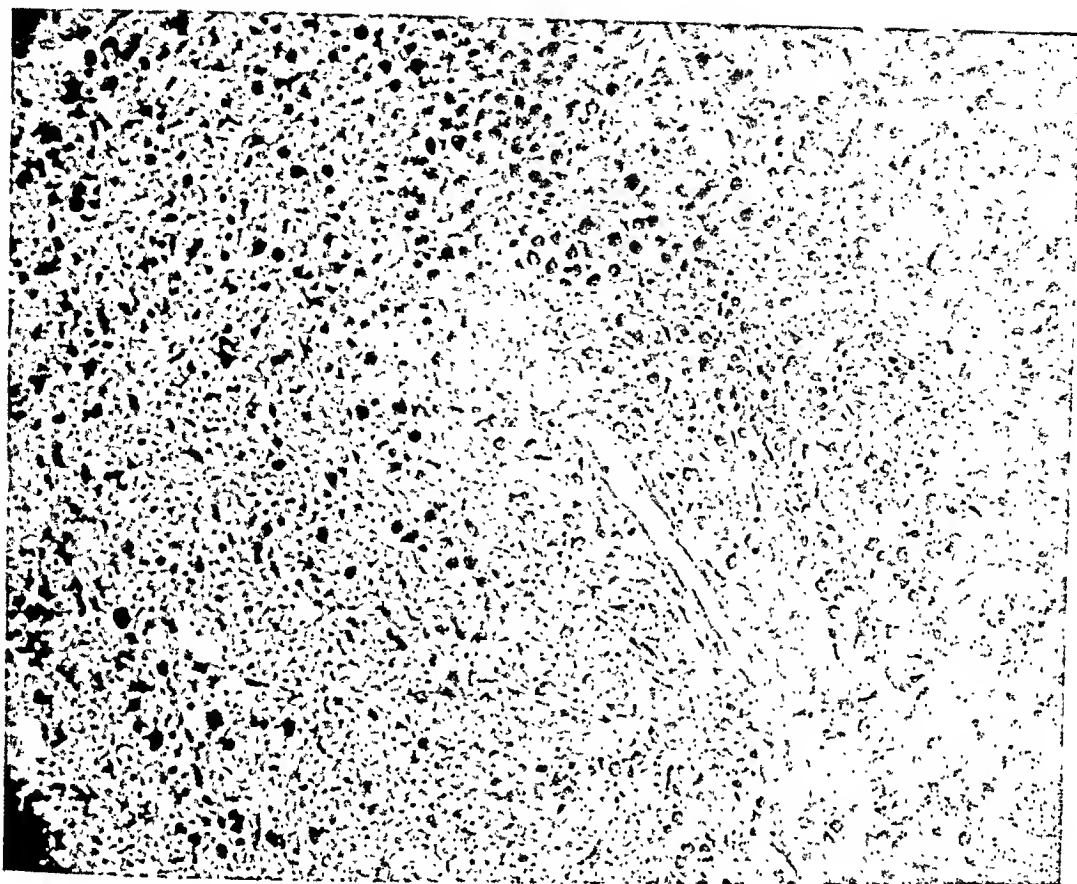
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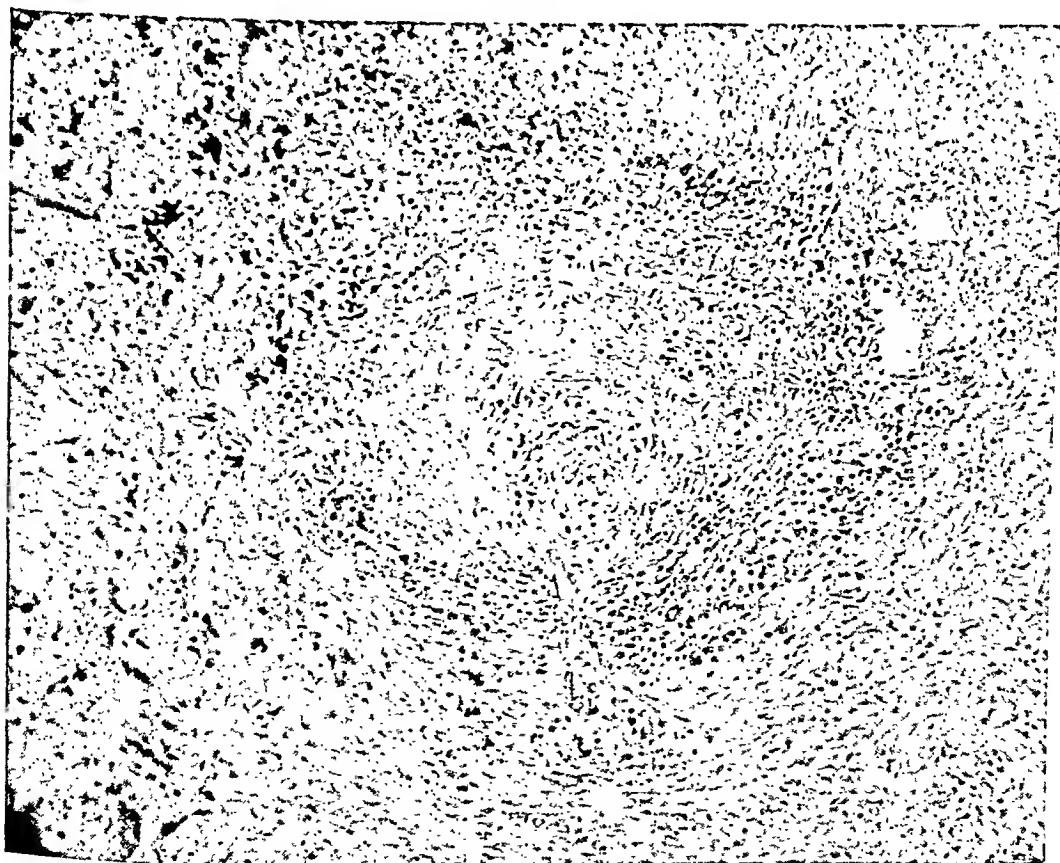
Disease of Swine Resembling Hodgkin's Disease

## PLATE 10

FIGS. 12 and 13. Alterations in the malpighian bodies of the spleen typical of the Hodgkin's-like disease of the hog are seen in Figure 12 ( $\times 285$ ) from case 2 and Figure 13 ( $\times 142$ ) from case 1. These lesions are the result of near healing of a typical granulomatous reaction in the lymphoid tissue surrounding the splenic arteriole, and are histologically identical with the most typical and regularly occurring splenic lesion of Hodgkin's disease.



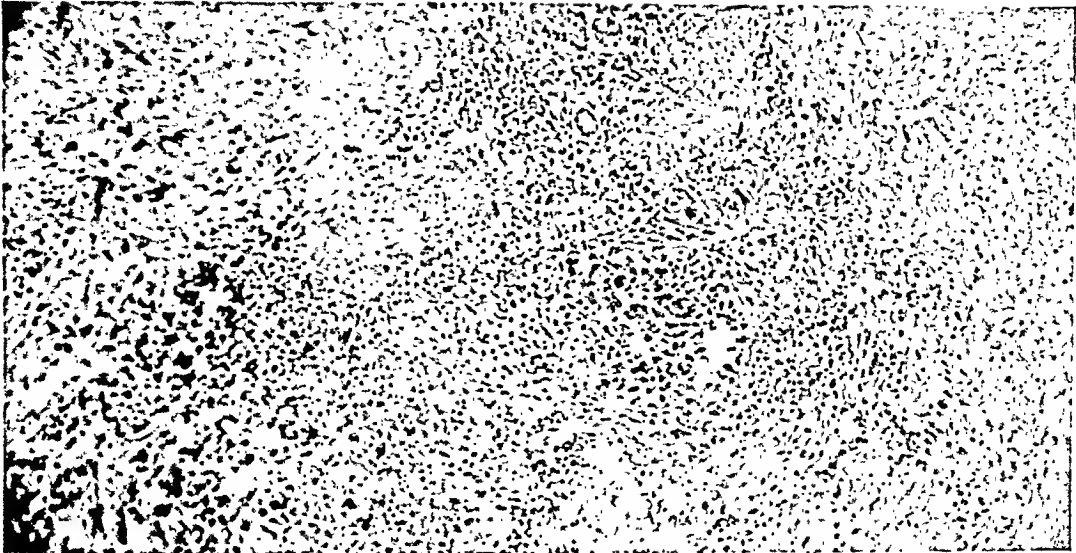
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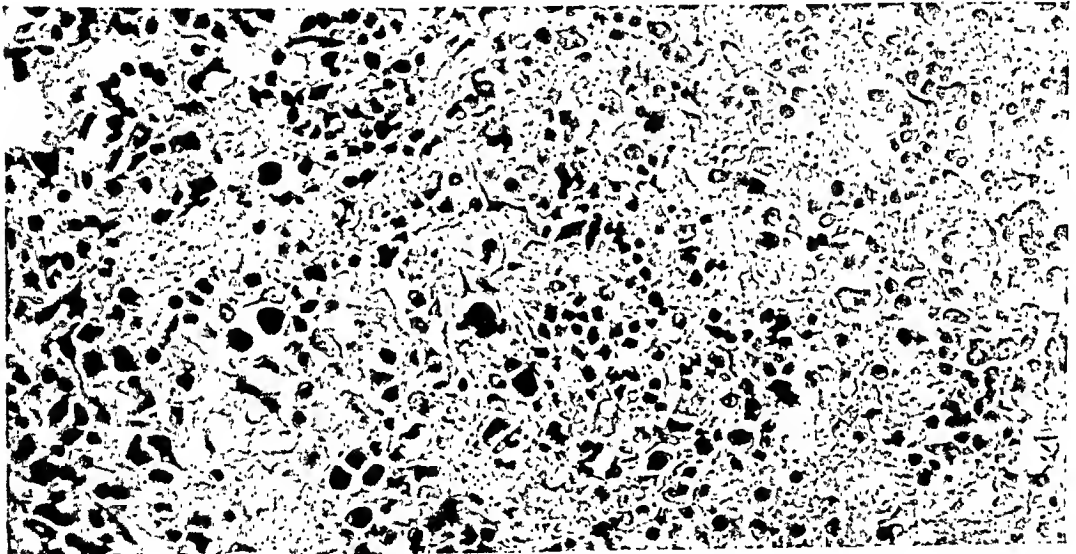
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## PLATE II

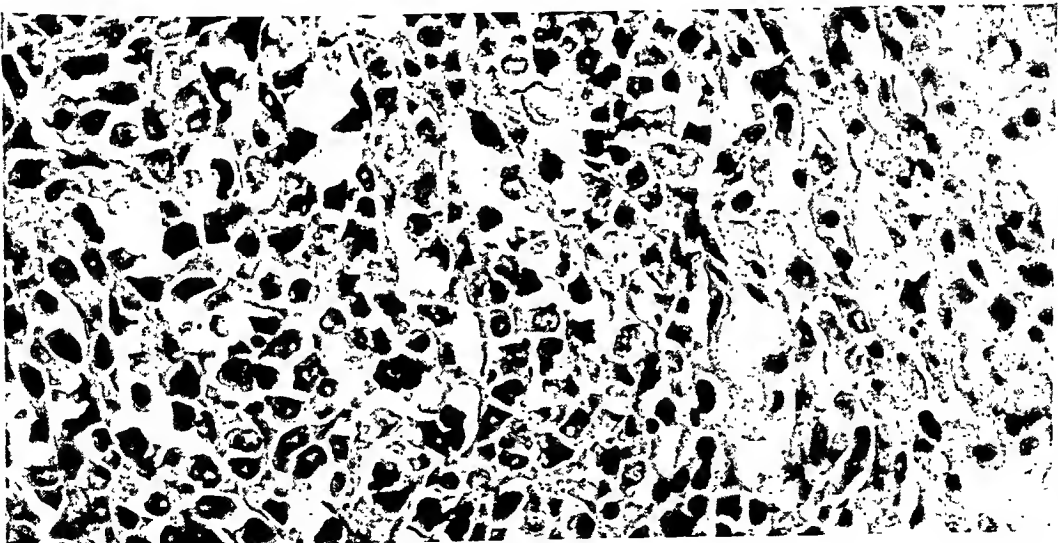
FIGS. 14, 15, and 16. Case 1. Typical lesions in the spleen. Figure 14 ( $\times 117$ ) shows confluent focal granulomatous lesions with adjacent fibrosis. Figure 15 ( $\times 345$ ) shows a more diffuse type of granulomatous reaction in which eosinophils, large mononuclear, lymphoid, and multinucleated giant cells are conspicuous. Figure 16 ( $\times 485$ ) represents the typical, focal, tumor-like, reticulo-endothelial proliferation which gives rise to gross nodules in the spleen such as those pictured in Figure 4. (See Fig. 3 for a low-power photograph of this spleen.) Lesions of the type shown in Figure 16 eventually undergo extensive fibrosis, the result being a hard nodule microscopically like that shown in Figure 19. An intermediate stage is represented in Figure 18.



14



15



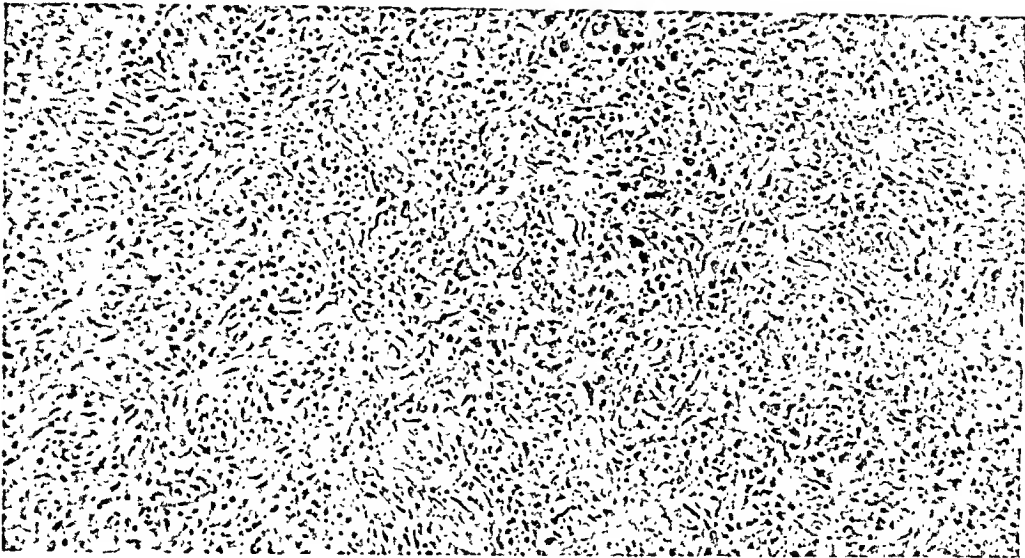
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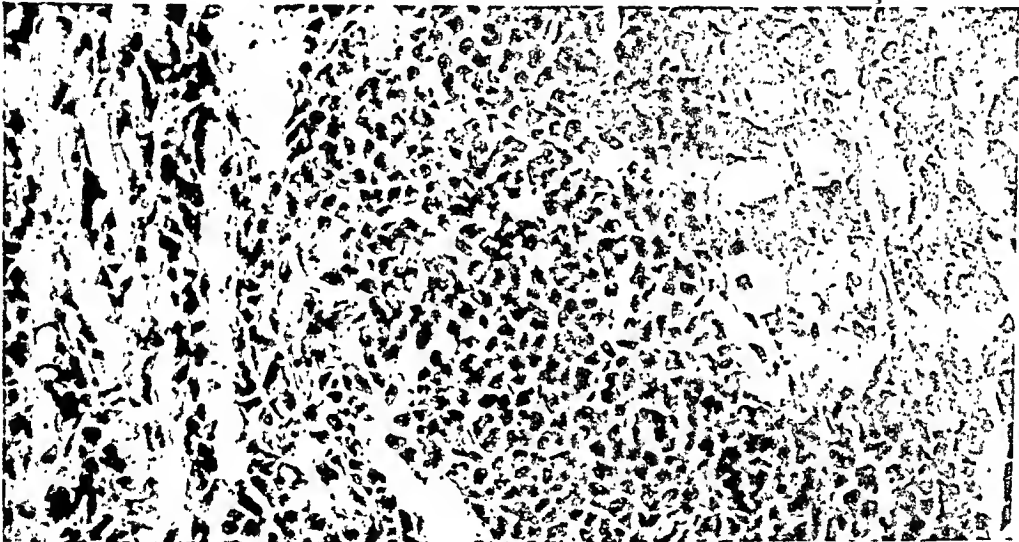
## PLATE 12

FIG. 17. Case 2. Photomicrograph showing the reticular scarring of the spleen with residual cellular components of the more diffuse granulomatous reaction typical of advanced lesions ( $\times 117$ ). A comparable view of the spleen of case 1 is shown in Figure 15. (See also Figs. 3 and 4.)

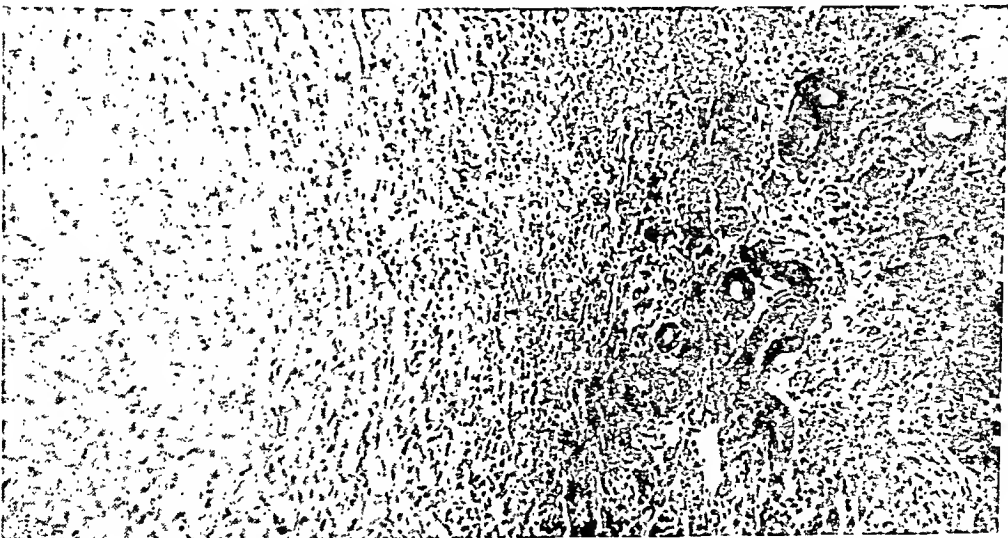
FIGS. 18 and 19. Case 3. Figure 18 shows the margin of a densely scarred nodule in the spleen ( $\times 315$ ). The cells at the center are eosinophilic leukocytes; the spaces in the tissue appeared to have been filled with crystals. Figure 19, a similar nodule at lower magnification ( $\times 147$ ), shows on the left the relatively acellular character of this old and presumably nearly healed granulomatous focus. It is possible to trace a direct relationship between the obvious granulomatous reaction (Figs. 14, 15, and 17), the focal tumor-like reticulo-endothelial proliferation (Fig. 16), and the fibrous healed lesions shown in these two photomicrographs.



17



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### PLATE 13

FIGS. 20 to 27. These figures show typical cytologic details of the more active stages of the granulomatous reaction which characterizes the lesion of the spleen in the Hodgkin's-like disease of the hog. Figure 20 is from case 2 (Figs. 4 and 17); Figure 21 is from case 6 (Fig. 5); and Figures 22 to 27 are from case 4 (Fig. 2). All are at the same magnification ( $\times 485$ ) and constitute a typical composite picture of the granulomatous lesion characteristic of the spleen.

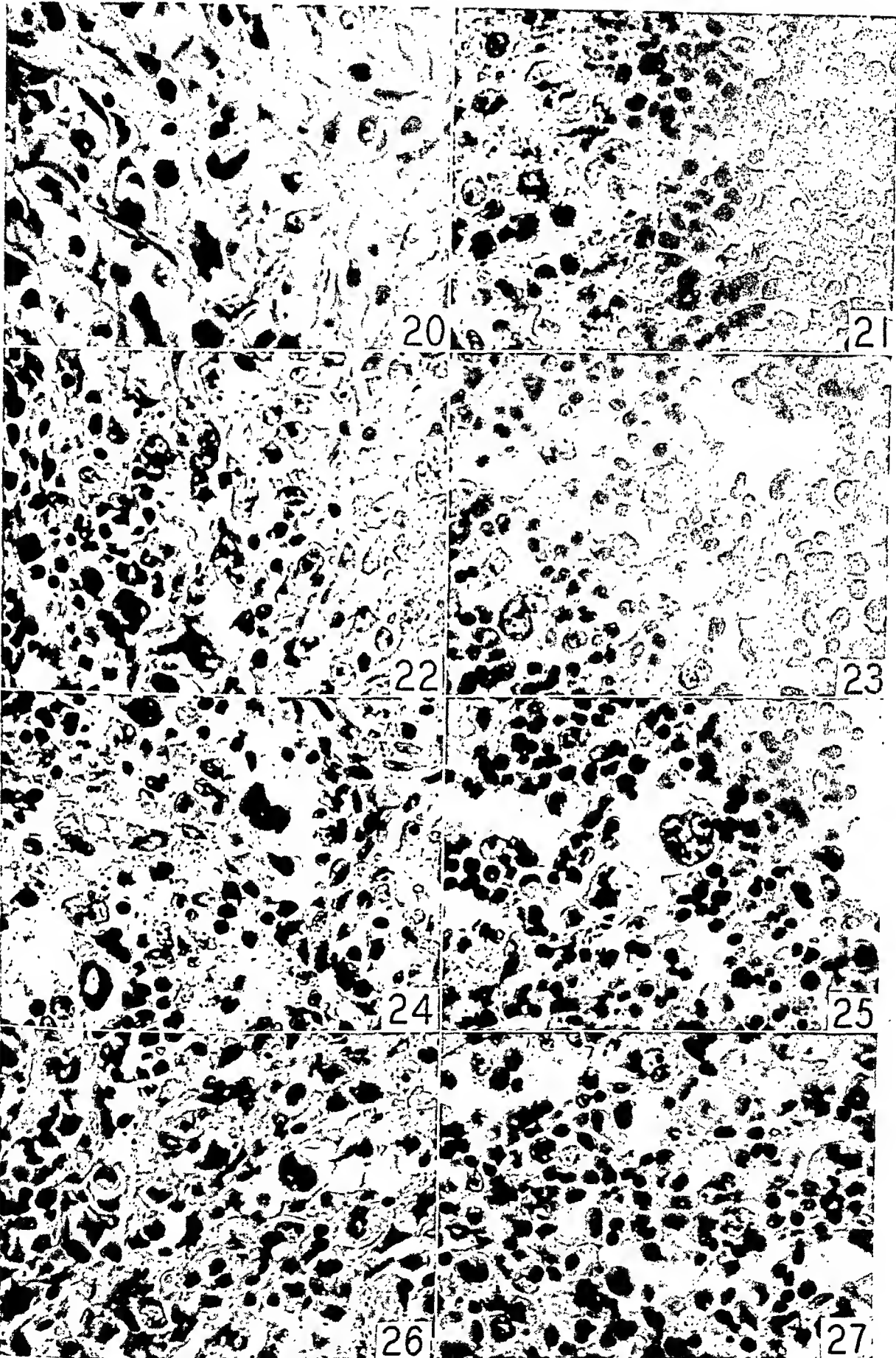
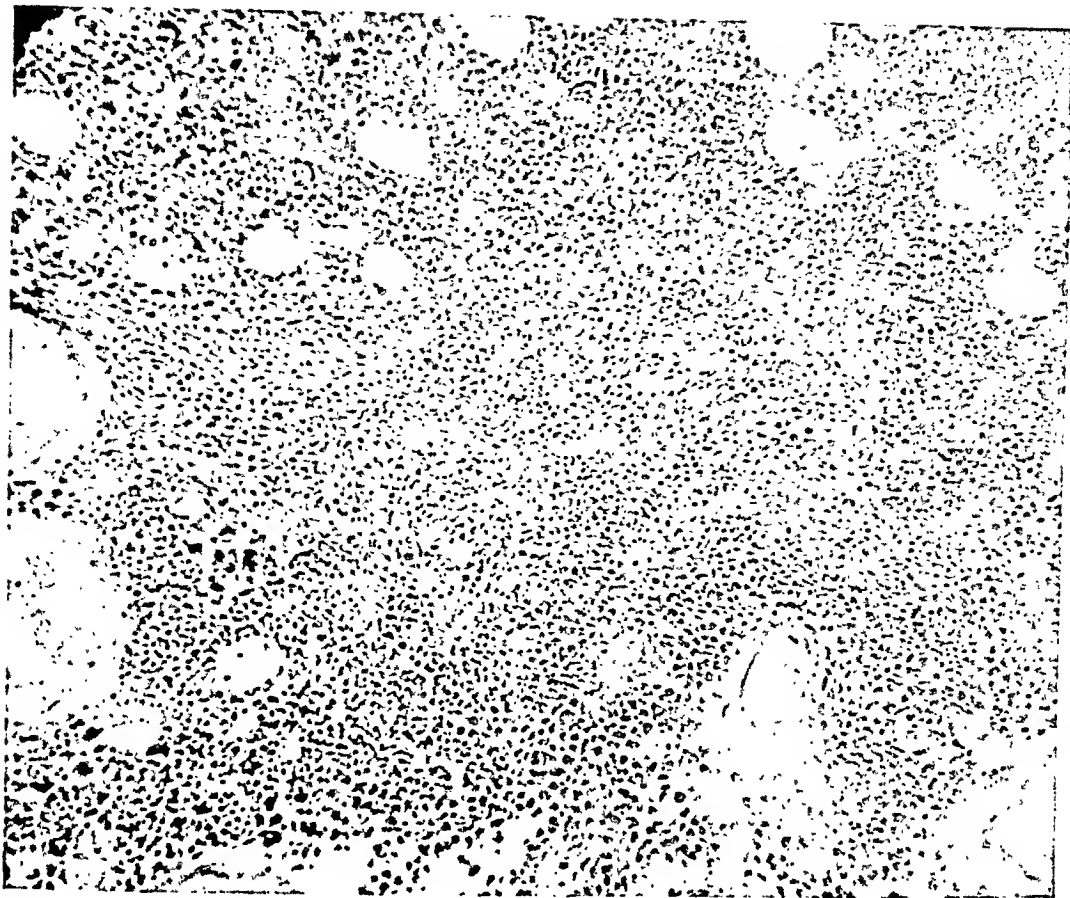
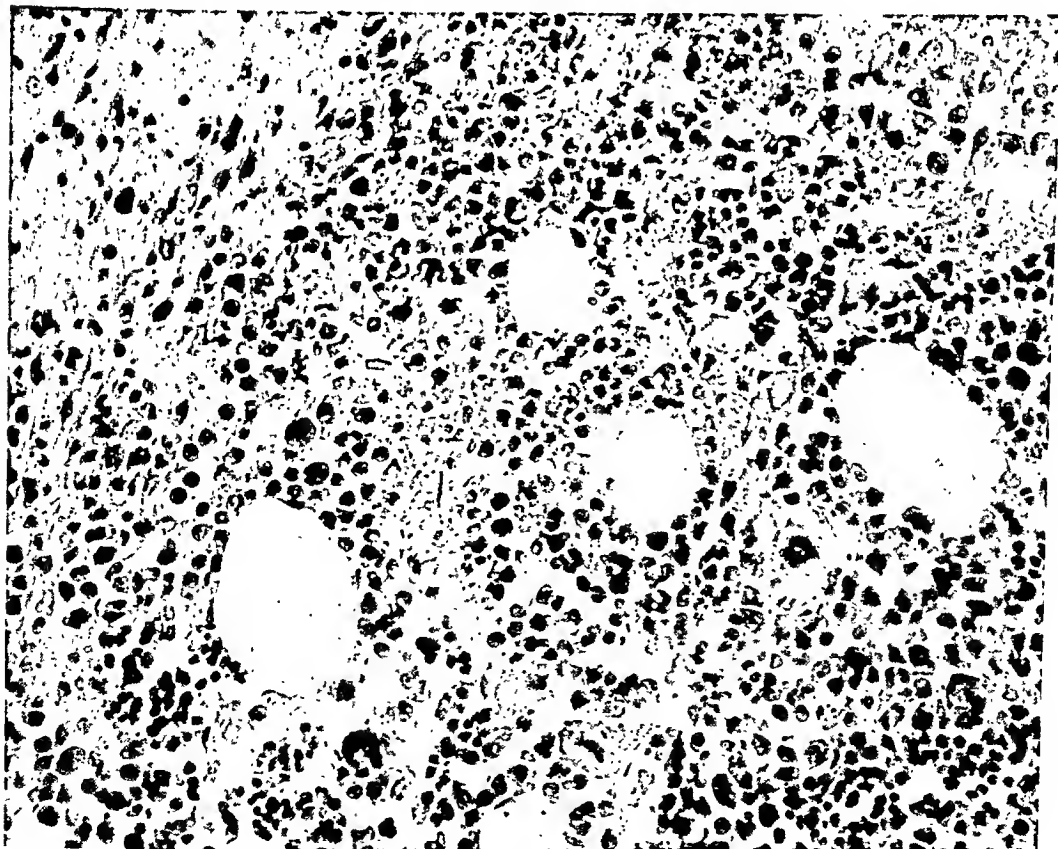


PLATE 14

FIGS. 28 and 29. Case 1. The bone marrow lesion is shown in a more active part of the typical granulomatous reaction (Fig. 28,  $\times 137$  and Fig. 29,  $\times 345$ ). (See also Fig. 6.)



28



29

PLATE 15

FIGS. 30 to 33. These figures show a composite picture of the granulomatous lesion of the lymph nodes (capsule at bottom, medulla at top) characteristic of the Hodgkin's-like disease of the hog. Figure 30 ( $\times 122$ ) is from case 1 (Fig. 1); Figures 31 ( $\times 137$ ) and 33 ( $\times 265$ ) are from case 4 (Fig. 2); and Figure 32 ( $\times 112$ ) is from case 5.

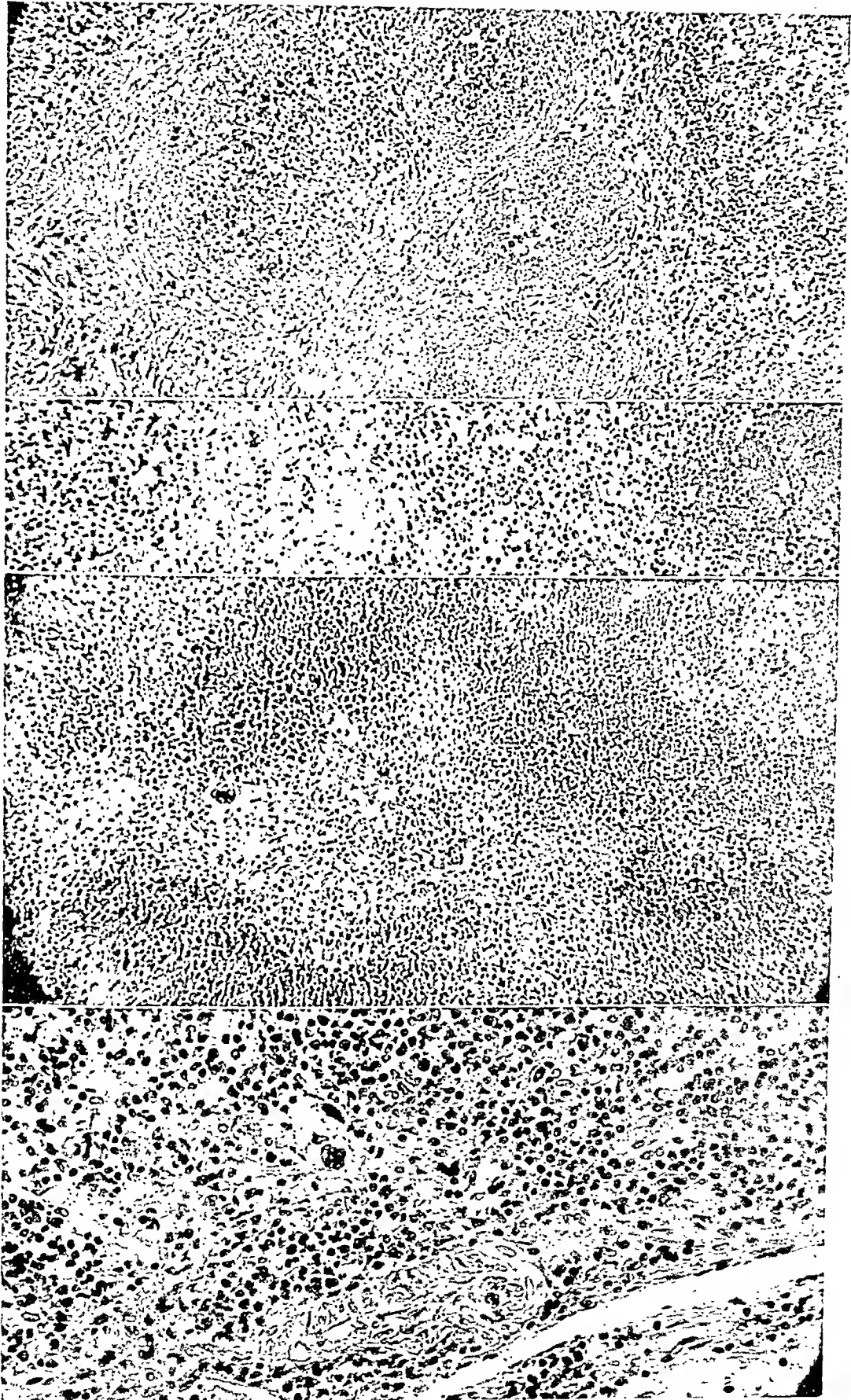
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## FAT EMBOLISM \*

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*(From the Army Institute of Pathology, Army Medical Museum, Washington 25, D.C.)*

Fat embolism, chiefly a pathologic curiosity in time of peace, is a problem of real clinical importance as a result of the inevitable increase in trauma associated with war. Although a considerable amount of animal experimentation has been carried out on the subject, interpretations of results are conflicting. Thus far no effective therapy is available. Consequently a clear understanding of the pathology of the condition, including its pathogenesis, is essential to minimize its occurrence and to determine more accurately its prognosis.

Fat embolism in man is due to fat gaining access to the circulation, either intrinsic fat freed through trauma or extrinsic fat or oil introduced into the organism for therapeutic or other purposes.

In an effort to determine the military importance of fat embolism and the factors favoring the development of the lesion, I have reviewed 100 consecutive cases of this condition in the files of the Army Institute of Pathology. These cases cover a period of approximately 4 years, from the time of large-scale mobilization to the latter part of 1944. The patients were chiefly young, healthy males. Ninety-one cases were due to trauma with fracture of one or more bones; 4 cases were due to blast injury without fracture; 3 to burns; and 1 each to pressure asphyxia without fracture, and to trauma from shell fragments.

The localization of the fractures in these 91 cases was notable. Some cases had fracture of only a single bone and others had multiple fractures, but the tibia, the femur, or both combined were involved in 82 per cent of the cases of fat embolism following fracture. This is perhaps due to the large reservoirs of fat they contain. In reviewing cases of fat embolism reported in the literature, the frequency of fractures of these bones is also apparent.

The means of trauma were varied. Motor vehicles produced injuries in the greatest number of cases (61). Horse, mule, street car, parachute, and glider each provided 1 case.

All but one of these cases occurred in males. The age distribution shown in Table I is about that which would be expected from army personnel, 59 per cent of the cases falling in the age group 18 to 25 inclusive.

\* This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the U. S. Navy. The opinions and views set forth in this article are those of the writer and are not to be considered as reflecting the policies of the Navy Department.

Received for publication, July 18, 1945.

The total amount of body fat has little relation to the occurrence of fat embolism. The state of nutrition or the body weight was specified in 85 cases. Of these, 62 were within normal limits and 23 were overweight. Physical selection would tend to keep the strikingly obese and the emaciated out of the service.

The blood pressure is not a factor. The blood pressure recorded before treatment ranged from none in cases of severe shock to 150/80 mm. Hg. The blood pressure after the onset of symptoms ranged from 0 to 160/90 mm. Hg in all but one case, in which the systolic pressure rose to 192 after the onset of symptoms.

Undoubtedly the state of the circulation is of some importance in influencing the severity of fat embolism, a patient with impaired circulation naturally feeling the effects of a given degree of pulmonary fat embolism more than a patient with good circulation.

The suggestion has been made that status lymphaticus is a predisposing factor in pulmonary embolism. In the course of some 7,000 autopsies, a number of them

TABLE I  
*Age Distribution of 100 Cases of Fatal Fat Embolism*

Years of age	Number
18 — 20	15
21 — 25	44
26 — 30	17
31 — 35	8
36 — 38	6
Unknown	10

cases of sudden death, I never have seen an instance of death that could be attributed to status lymphaticus. In light of this and of the work of Farber,<sup>1</sup> Boyd,<sup>2</sup> and others, I doubt that such a diagnosis is ever justified.

There is virtually complete agreement as to the essentials for entry of the embolic fat into the circulation: mobilized fat, veins disrupted and patent, and local pressure.<sup>3,4</sup> Particularly in bone trauma are these factors operative. Fat is set free from the marrow cells. Disruption of veins and venules without opportunity for collapse of their walls is the rule. As hemorrhage, edema, and exudation develop about the fracture, the local pressure is greater than the venous pressure. Consequently it is easy for fat to find its way into the disrupted patent venous channels. Any mobilization of a fracture after local swelling has occurred is fraught with hazard, as a considerable degree of local pressure then exists. Disturbance of the region may dislodge the fibrin occluding disrupted veins, opening them to the free fat present.

In concussions or burns, fat embolism is somewhat more difficult to explain. In marked concussions, as bomb explosion, the extensive disruption of the adipose tissues will very easily force fat into the circulation. In the case of burns, the death of fat cells in the panniculus

may liberate fat and local edema beneath an eschar or a pressure bandage will tend to force the fat into the capillary or venous circulation.

There has been much discussion of the importance of the amount of fat in the blood in relation to embolism. However, the physical state of the fat is probably much more important than is its amount. The normal blood fat varies from 0.6 to 0.7 per cent.<sup>5</sup> The mere presence of even very large amounts of fat in the blood stream will not produce embolism. Thus in diabetic coma the total blood lipid may reach 20 per cent. Although rare instances have been reported of death from fat embolism in diabetic coma, I have not seen such a case among several hundred patients with diabetic coma. The fat is finely divided as chylomicrons and the number of these tends to increase with the total lipid.<sup>6</sup> So long as the fat remains thus divided it is not dangerous. There is no convincing evidence that chylomicrons coalesce to form masses able to produce embolism. Wilson and Salisbury<sup>7</sup> suggested that dark-field study of the blood is worth while to determine the size of the chylomicrons in cases of suspected fat embolism. They stated that globules up to  $12\ \mu$  in diameter will go through the pulmonary capillaries.

Curiously enough, in fat embolism the level of total blood fat may not be at all high. While blood fat determinations were run in only a few of the cases in the present series, these were not abnormal.

The fat present in embolism is evidently still fluid, as it can be forced into various shapes to conform to the vascular beds and often can penetrate the finest capillaries. If autopsy is done when the body is still warm, the fat is fluid.

The estimates of the minimal lethal amount of fat entering the circulation of man range from 12 gm.<sup>8</sup> to 120 cc.<sup>9</sup> However, Lehman and Moore, from whose work the larger value is quoted, carried out their experiments on the dog, using cottonseed oil. Since there are wide ranges in tolerance to damage of various types between man and animals, it is unsafe to argue on the basis of their data, which are not in keeping with clinical experience. As little as 2 cc. of paraffin oil used as a lubricant in urethral dilatation has been blamed for embolic death, but the evidence here was not decisive.

Harris, Perrett, and MacLachlin<sup>10</sup> found the minimal lethal dose of human fat for rabbits to be 0.9 cc. per kg. when given in one dose. A much larger amount could be administered without producing death if the dose was divided. Hydrolyzed human fat was much more toxic, the minimal dose being 0.07 cc. per kg. They believed that the tissue lipase splits the fat and that the fatty acids produced by hydrolysis irritate the tissues.

In several cases which I studied the reaction to Nile blue sulfate showed the fat present in even markedly edematous lungs to be neutral fat and not hydrolyzed (Fig. 3).

Vance<sup>11</sup> found 4 to 60 cc. of fat at the site of fracture in bones of the lower extremity. Flick and Traum<sup>12</sup> studied the fat content of arterial and venous blood soon after trauma and found significant increases as early as 10 minutes after injury. Scriba<sup>13</sup> found fat present in the urine of 80 per cent of his cases 2 to 6 days after trauma to bone. Occasionally the fat will disappear from the urine transiently, to reappear later.

The cases studied in this series demonstrate that the fracture of a single bone may, under appropriate circumstances, release enough fluid fat into the circulation at a sufficiently rapid rate to produce death.

The highest level of blood lipid that I have seen recorded was in a case of carbon tetrachloride poisoning reported by MacMahon and Weiss.<sup>14</sup> In this case the blood obtained from the pulmonary artery at autopsy showed 64 per cent total lipid by volume and the blood from the upper end of the inferior vena cava showed 25 per cent. Specimens of blood taken from other portions of the body showed no gross fat. Extensive necrosis of the hepatic cells caused by carbon tetrachloride liberated large amounts of fat in the disorganized hepatic tissue and it was thus easy for it to enter the sinusoids and hepatic veins. The sudan stain of sections of the lungs showed many of the smaller branches of the pulmonary artery completely filled with fat, and congestion and focal hemorrhages. Sudan stains of the other organs showed minute droplets of fat in the vessels of the systemic circulation. In addition to visible fat within the glomerular capillaries, fatty casts were present in the collecting tubules. While death was not ascribed by the authors to pulmonary fat embolism, in view of the high degree of cholemia and the extensive necrosis of the liver, one cannot help but wonder if embolism may not have been an important factor.

Figures vary greatly as to the incidence of fat embolism. Darrach<sup>15</sup> reported 2 cases of fat embolism among 12,000 fractures treated at the Presbyterian Hospital in New York. Gröndahl<sup>16</sup> reported 1 case of fatal cerebral fat embolism among 1,026 fractures. Wilson and Salisbury<sup>7</sup> reported 8 cases, 6 of which were fatal, among 1,000 battle casualties of World War II. Robb-Smith<sup>17</sup> studied 789 consecutive (bombing) accidents, of which 125 were fatal. Forty-one of the fatal cases showed gross pulmonary fat emboli and in 29 pulmonary embolism was a major factor in the deaths. In the group of 125 fatal cases, 12 showed no bony injury and 6 of these were due to fat embolism. On the basis of this he suggested that pulmonary embolism

may be an important factor in death from bombings. Wakeley<sup>18</sup> found fat embolism present to varying degrees in 40 per cent of fatally burned cases. In 100 consecutive autopsies, Wright<sup>19</sup> checked on the presence of fat in the pulmonary vessels. In 52 some degree of fat was present on microscopic examination. Globules of fat were present in every capillary in 3 cases, a globule in every low-power field appeared in 1, and the remainder showed only slight amounts of fat.

While attempts have been made to relate specific symptoms to specific localization in the vascular bed, these have not been very satisfactory: first, because with a severe degree of fat embolism more than one viscus is usually involved and, second, because the pulmonary edema and congestion associated with fat embolism lead to a very frequent masking of other symptoms by the obvious respiratory embarrassment and anoxia.

Of special interest is the existence of a period free from symptoms following trauma in many cases. This free period varies and its close is frequently foreshadowed by a feeling of marked apprehension on the part of the patient. This feeling of apprehension has also been reported in cases of pulmonary embolism of thrombotic or other origin.

Fever is of inconstant occurrence and may be related to disturbance of cerebral function, to infection in the wound, or to the development of pneumonia in the edematous lung. The diagnosis is an especially difficult one at which to arrive and frequently autopsy alone will reveal the cause of death. Physical examination or roentgenography is of little or no help. The occurrence of pulmonary edema in a case of recent trauma should lead to the suspicion of fat embolism and at the end of 36 hours examination of the sputum for fat globules may prove positive. Lipuria may be present but is a rare finding. Examination of the blood for fat is probably of little value.

The gross and microscopic evidences present a wide range of variation. In cases of rapid death there may be nothing apparent grossly beyond an excess of oily droplets in the pulmonary circulation. In the acute cases there is usually accompanying pulmonary edema and in the brain there may be petechial hemorrhages, as a rule perivascular. In cases of longer duration there may be consolidation of the lungs in addition to edema and hemorrhage; in the brain there may be small foci of softening, often surrounded by a hemorrhagic zone. In the pulmonary forms, evidences of asphyxia may be present: marked congestion of the chest, neck, and head; petechial hemorrhages, which occur in order of frequency in the pericardium, the conjunctivae, and the pleurae. Petechial hemorrhages may also appear in various tissues as a result of occlusion of small vessels by the droplets.

On microscopic examination, fat droplets are detected in the small vessels and capillaries of most tissues. These predominate in the lungs and in the glomerular tufts of the kidneys. The size of the droplets varies from a few  $\mu$  in diameter to 1 cm. or more. The shape of the occluding droplets varies from spherical to elongated cylinders, the shape being determined by the three factors of vessel caliber, surface tension, and circulatory pressure. On becoming freed, by section of the tissue in which they lie, they coalesce rather freely, indicating that no definite membrane is formed about the fat but rather that it is merely coarsely and irregularly suspended in the blood. Nothing that I have seen in these sections would indicate that there has been any appreciable breakdown of the fat or that the fat was in any way toxic to either vascular endothelium or formed elements of the blood.

To detect fat embolism microscopically in routine autopsy material it is well in the ordinarily stained paraffin section to examine the glomeruli and the lungs. If there are a considerable number of vacuoles within the capillaries of the tuft or if some of the alveolar capillaries show vacuoles, it is well to do a fat stain on frozen sections of fresh or autopsy material. It is important that the section should be cut rather thicker than is usually the case, say 30 to 50  $\mu$ , inasmuch as the droplets of fat are easily dislodged.

Figure 1, a routine paraffin section, is an example of the extreme degree of vacuolization that can occur in a glomerular tuft due to the lodging of fat in it. Figure 2 shows the sudan stain on a formalin-fixed, frozen section. Figure 7 is a routine paraffin section of the lung with readily apparent vacuoles within the pulmonary vessels. Figure 4 is a frozen section counterpart, stained specifically for fat, showing occlusion of capillaries.

Bürger<sup>20</sup> suggested distinguishing three forms of the pulmonary type of fat embolism: (1) the peracute, where death occurs in a few seconds; (2) the acute, in which the symptoms appear immediately after injury and death occurs in hours to days (the type usually confused with pulmonary edema); (3) the subacute, where a free period intervenes between trauma and the onset of symptoms. The cases studied by me can be grouped into these three types (Table II).

The average time from the onset of symptoms to death in this series was 53 hours and the longest duration, 25 days.

In 3 cases of this series, the origin of the fat from bone was obvious since the fat in the pulmonary vessels was accompanied by fragments of bone marrow.

Although Bergmann<sup>21</sup> noted in 1863 that acute pulmonary edema was often associated with fat embolism, inadequate emphasis has been

placed on this point. The present study brings out the importance of pulmonary edema, often combined with congestion or focal hemorrhage, in the clinical course. Occlusion of large or small branches of the pulmonary artery or pulmonary capillaries leads inevitably to impairment of the endothelium and its supporting cells with increased endothelial permeability so that large amounts of fluid may escape into the lungs.

No sooner has an appreciable amount of the pulmonary circulation been impaired than partial local anoxia begins to develop. Infarction or necrosis of pulmonary tissue seldom occurs since even in extensive and prolonged fat embolism there is a sufficient degree of blood flow

kept up by the bronchial arteries and uninvolved portions of the pulmonary tree to prevent actual cell death. However, focal or petechial hemorrhages may appear at regions of vascular occlusion. The local anoxia induced soon leads to an increased permea-

TABLE II  
*Classification of Cases of Fat Embolism*

Peracute cases	9
Acute	17
Subacute	58
Undetermined	16

bility of the vascular and alveolar walls so that escape of intravascular fluid occurs into the pulmonary tissues and alveoli. This edema may become exceedingly marked (Fig. 7), interfering further with aeration and thus establishing a vicious circle.

The average weight of the lungs in these cases, when specified, was 1600 gm. As a general rule, the few cases in which the lungs were relatively free from fluid were peracute or acute cases of short duration in which sufficient time for the development of edema had not been present.

There has been some speculation as to the induction of arterial spasm by the presence of the fat and further production of anoxia by this means. There is no satisfactory experimental evidence for this and the general experience with anoxia in shock and other conditions is that there is a tendency to vasodilatation in the viscera rather than vasoconstriction. The present data do not support the view that such arterial spasm occurs.

Within a few hours after lodging, the fat in the pulmonary circulation begins to gain access to the alveoli and is visible within them, usually as small droplets ranging up to 15 to 20  $\mu$  in diameter. These at first are merely dispersed in the edema fluid which accumulates within the alveoli (Fig. 8), but later they are to some degree phagocytized by mononuclear cells, in some instances they become incorporated as vacuoles within the substance of foreign body giant cells. In



view of this phagocytosis, it is not surprising that in those patients living several days after the onset of symptoms of embolism, one will encounter some phagocytized fat in the pulmonary parenchyma. In several of the cases in which the patients had lived for some days, I found fat within phagocytes in mediastinal lymph nodes.

After some 36 hours fat may be observed in the sputum.<sup>22</sup> Staining the sputum of a case of supposed pulmonary edema for fat may disclose fat droplets and thus establish the diagnosis.

Undoubtedly some of the fat may escape from the vessels by actual disruption of their walls. Much of it, however, is present in alveoli in which the capillaries are intact. The cement substance of endothelium and of many other tissues has a lipoidal content which may permit fatty substances, under suitable circumstances, to pass through an intact vessel wall.

The severity of pulmonary fat embolism in the present series of cases is given in Table III, which shows that in nearly two-thirds of the cases, lodging of fat to a severe degree occurred. Thirty-four patients presented respiratory symptoms as the most striking clinical feature.

TABLE III  
*Severity of Pulmonary Fat Embolism*

Fat			Marrow
+	++	+++	
4	34	62	3

While it has been suggested<sup>23</sup> that there is marked renal damage with fat embolism, there is no evidence to support this in my material. In spite of the almost constant involvement of the renal glomerular capillaries (Table IV), the clinical significance is minimal. There is little need to alter the statement made by Warthin<sup>3</sup> in his classic monograph that "Nephritis has never been mentioned as a complication." One can but wonder why the renal glomeruli do not show evidence of damage even when there is virtually complete occlusion by fat of the afferent arterioles as well as all of the capillaries in the glomerular tufts (Figs. 1 and 2). Although involvement of the glomerulus is a frequent feature of fat embolism, I did not encounter in this series a single death due to impairment of renal function. Evidently, either death comes before noticeable renal change can occur, or the damage, if any, is soon repaired. Perhaps the intermittent character of the renal glomerular circulation may have prepared the cells of the tuft to withstand periods of anoxia, and perhaps some tufts, nonfunctioning at the time fat droplets were plentiful in the blood stream, escape embolism.

Lipuria as a means of demonstrating fat embolism is not reliable. It rarely develops before the fourth day. In the majority of the cases in the present series, the urine was not examined for fat. Many pa-

tients died prior to the time when lipuria might have been expected. In some cases it was possible after death to demonstrate fat droplets within the lumen of the tubules (Fig. 5). Rarely, numerous fat droplets were present in the tubular epithelium.

Twenty-four patients died with symptoms predominantly cerebral. The gross and microscopic lesions of the brain have been well studied in the past.<sup>4</sup> The reported findings are confirmed by the present observations. The classical picture is the appearance of small petechial hemorrhages in the leptomeninges as well as throughout the brain, and minute foci of softening scattered indiscriminately in the cortex and

TABLE IV  
*Renal Abnormalities with Fat Embolism*

	Fat in glomeruli				Fat in tubules	Tubular degeneration
	None	+	++	+++		
Number of cases	17	11	15	20	4	0

medulla. There are varying degrees of minute focal hemorrhage. Schmidt<sup>24</sup> used the rather good descriptive term of "purpura cerebri" in this condition. The transition from the gray matter to the white may be indistinct. At times the emboli excite no reaction (Fig. 6).

Microscopically, early vascular occlusion may occur without reaction, or there may be perivascular hemorrhage (Fig. 9), or chiefly perivascular edema (Fig. 10). Lesions of longer duration may present small foci of degeneration (Fig. 11) adjacent to a vessel occluded by fat, and sometimes there are small anemic infarcts with a hemorrhagic periphery similar to that seen in anemic infarcts otherwise produced. A fair number of polymorphonuclear leukocytes are present in the earlier stages of the lesion. These gradually degenerate and mononuclear phagocytes take their place. The infarcts may be anemic in the gray matter, hemorrhagic in the white.<sup>25</sup> Changes have been described in the neurons themselves but it is very difficult to differentiate between the effect of the general anoxia on them and the effect of local embolic occlusion. Advanced lesions are rarely seen since death, if it occurs, tends to appear fairly soon after cerebral involvement.

Trauma to the head had occurred in 33 cases. There was no correlation between this and fat embolism of the brain.

While fat embolism of the central nervous system occurs frequently (31 of 100 cases in the present series), there is no correlation between it and pulmonary embolism other than the fact that cerebral embolism, of course, cannot occur without some degree of pulmonary involve-

ment. Cerebral fat embolism may be found at autopsy in a slight or moderate degree without there having been presented any evidence of its occurrence during the life of the patient. In this present series, a slight amount of fat was present in 7 cases, a moderate amount in 13, and severe involvement in 11.

A rapid rise in temperature without evidence of pneumonia suggests that the brain has been involved by the embolic process. Among the few patients with recognized fat embolism who have recovered, residual mental or neurologic changes have not been noticed. The character of the lesions as seen in some fatal cases, however, would suggest that such changes will eventually be found.

Since fat which passes through the pulmonary circulation or through a patent foramen ovale may lodge anywhere in the systemic circulation, one finds at times very widespread emboli. It is questionable whether embolic involvement of coronary vessels may be a significant factor in producing death. In cases where the coronary arteries are heavily involved there is much embolism elsewhere. In the present series I encountered 8 cases in which an apparently significant degree of coronary involvement was present. One case of the series showed a patent foramen ovale, but the clinical picture and the distribution of fat did not differ from those of the others.

Owing to the lodging of small emboli in the vessels of the skin or mucous membranes, petechiae may develop<sup>26</sup> (Fig. 12). At autopsy, of course, pericardial and pleural petechiae associated with the anoxia produced by the pulmonary embolism are of frequent occurrence.

Rarely, changes may be noted in the eyegrounds, but these are usually degenerative in type<sup>27</sup> rather than hemorrhagic, and probably result from embolic occlusion of the nutrient vessels.

Unfortunately, the nature of the condition is such that no specific therapy for fat embolism exists, and such measures as are applied are usually futile. In general, supportive treatment is about all that can be attempted.<sup>28</sup> Once the pulmonary form has been recognized, the use of oxygen may be advantageous to prevent further development of anoxia and pulmonary edema.

In the way of prophylaxis one can do little but reiterate the suggestions already made many times that fractures be immobilized to the greatest possible degree and that any manipulation of them be carried out either before appreciable local swelling has occurred or after it has subsided.

#### SUMMARY AND CONCLUSIONS

1. The pathologic findings in 100 fatal cases of fat embolism have been summarized. Ninety-one cases followed fracture of one or more bones.

2. Fractures of femur, tibia, or both, either alone or combined with fractures of other bones, were antecedent to fat embolism in 82 per cent.
3. Fat embolism may also follow extensive trauma to soft parts; for example, through bombing, burning, or crushing.
4. Fat embolism is not due to aggregation of circulating fat, but to quantities of fat set free from the bodily deposits, or rarely, to fats introduced from without.
5. Fat embolism in the present series of cases produced instant death (peracute form), in 9, death following symptoms of immediate origin (acute form) in 17, or death following symptoms developing after a free period (subacute form) in 58 cases.
6. The occurrence of fat embolism is apparently independent of the nutritional state.
7. A free period in which the patient may be apprehensive may precede the development of symptoms.
8. Fat embolism may be recognized clinically by the development of pulmonary or cerebral symptoms following (a) a fracture or extensive injury of soft parts; (b) manipulation of a fracture. After some time, lipuria or fat droplets in the sputum may aid in establishing the diagnosis.
9. Although renal fat embolism may be marked, renal failure is not a cause of death.
10. In many cases fat embolism will be missed as a cause of death unless it is searched for consistently.
11. In autopsy material the presence of fat may be easily checked by watching for vacuoles in the glomerular tufts or in the lung parenchyma in routine sections.
12. Therapy is usually unavailing and is best directed toward the prevention of anoxia.

I am indebted to the staff of the Army Medical Museum for help, without which the publication of this study would have been impossible. In particular, I wish to thank the Photographic Department which prepared all of the illustrations.

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## DESCRIPTION OF PLATES

### PLATE 16

FIG. 1. Paraffin section of glomerular tuft showing vacuolization of capillaries and arteriole due to embolic fat. Case 118020. Hematoxylin and eosin stain.  $\times 500$ .

FIG. 2. Frozen section of glomerular tuft showing vacuolization of capillaries and arteriole due to embolic fat. Case 117306. Sudan IV stain.  $\times 500$ .

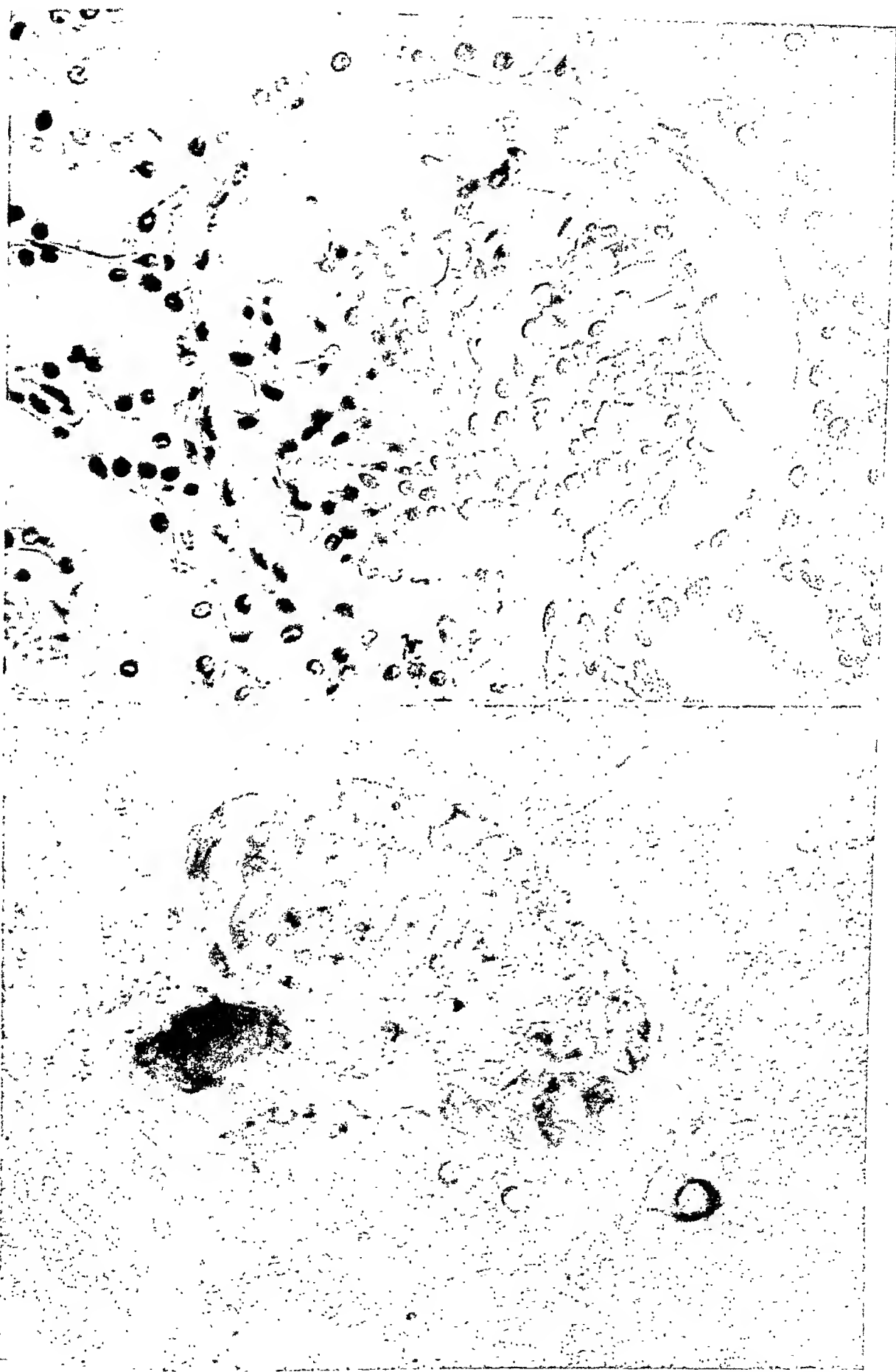
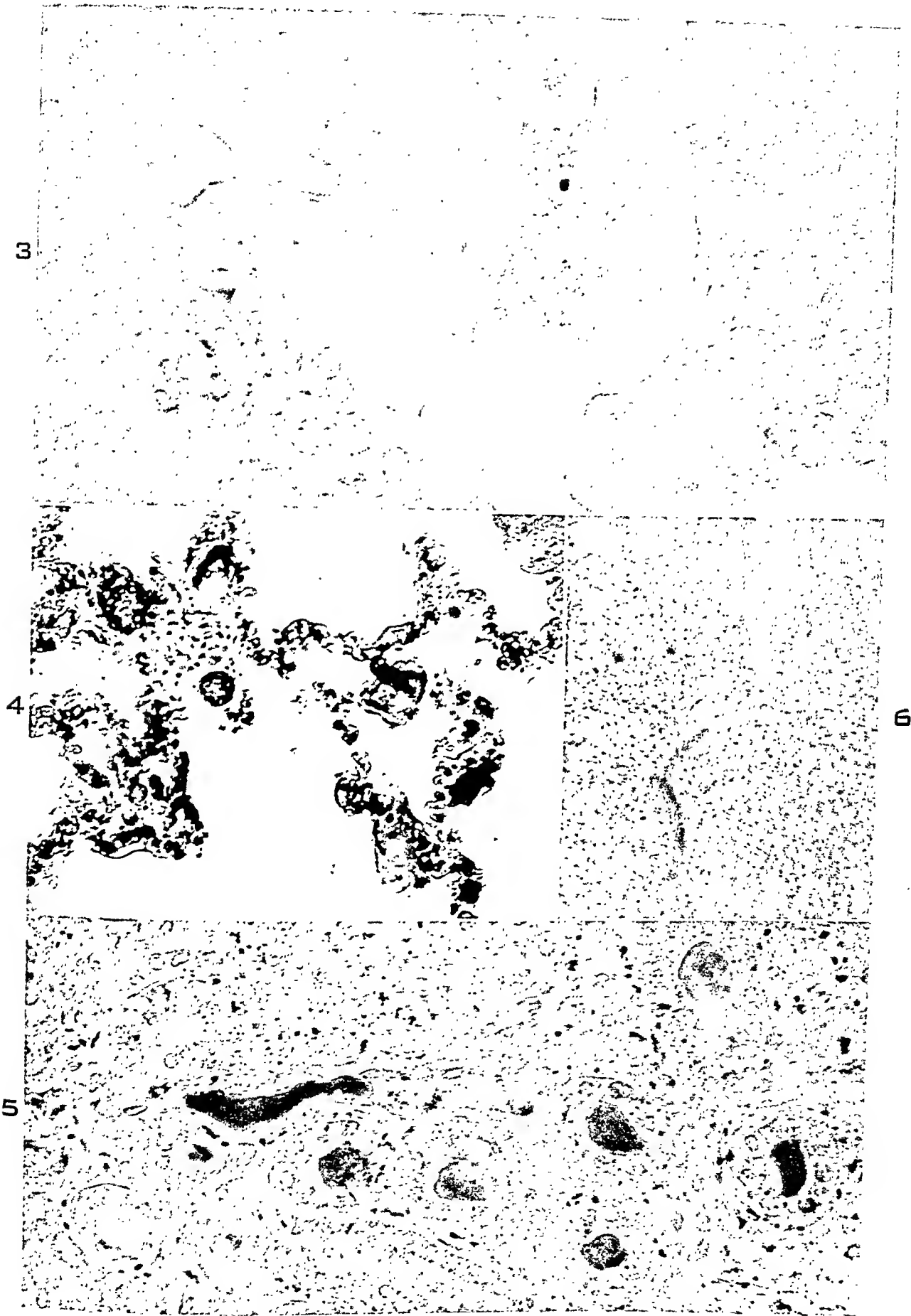


PLATE 17

- FIG. 3. Pulmonary fat embolism showing neutral fat in blood vessels and in edematous alveoli. Case 118020. Nile blue sulfate stain.  $\times 500$ .
- FIG. 4. Fat occluding alveolar capillaries. Case 114289. Sudan IV stain.  $\times 160$ .
- FIG. 5. Fat present within the lumina of renal tubules. There is no evidence of injury of the tubular epithelium. Case 117306. Sudan IV stain.  $\times 500$ .
- FIG. 6. Embolic occlusion by fat of capillaries in the white matter of the cerebrum. No reaction is present. Case 117306. Sudan IV stain.  $\times 230$ .





# PLATE 18

FIG. 7. Fat droplets within pulmonary arteriole, showing alveolar edema and small fat droplets. Case 118020. Hematoxylin and eosin stain.  $\times 145$ .

FIG. 8. Fat droplets in pulmonary vessels and in edema fluid within alveoli. Case 118020. Sudan IV stain.  $\times 145$ .

FIG. 9. Perivascular hemorrhage about arterioles occluded by fat. Case 99570. Hematoxylin and eosin stain.  $\times 145$ .

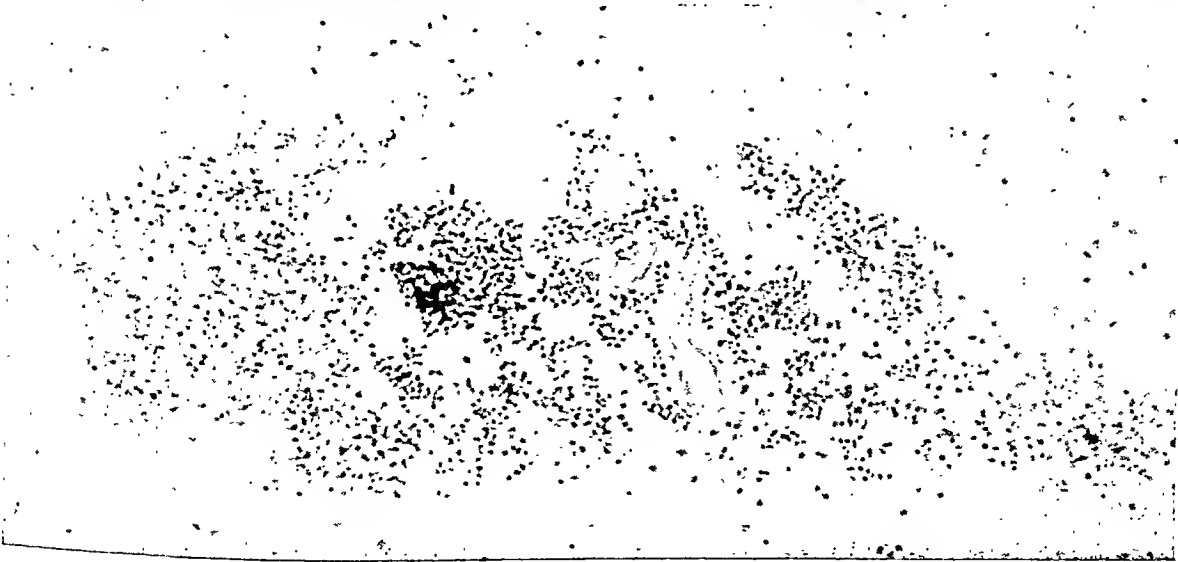
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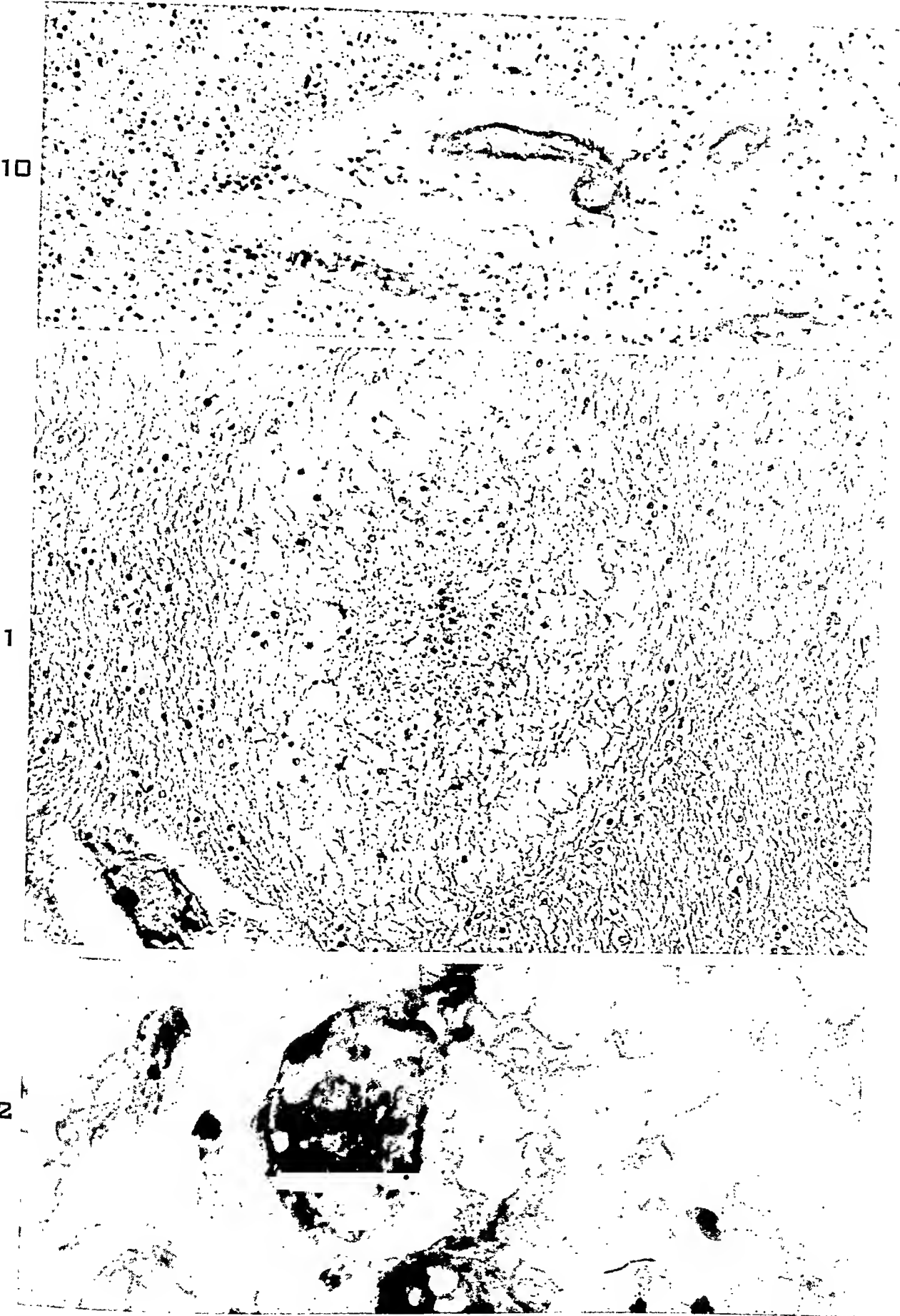
Fat Embolism

PLATE 19

FIG. 10. Perivascular edema in the white matter of the cerebrum secondary to fat emboli. Case 118020. Hematoxylin and eosin stain.  $\times 145$ .

FIG. 11. Focal demyelination in the white matter of the cerebrum, resulting from embolic occlusion of small arterioles by fat. Case 100989. Hematoxylin and eosin stain.  $\times 230$ .

FIG. 12. Capillaries in panniculus adiposus, partly occluded by fat droplets. This case showed petechial hemorrhages of the skin. Case 117306. Hematoxylin and eosin stain.  $\times 500$ .



Warren

Fat Embolism



# GROWTH OF THE RICKETTSIAE OF TSUTSUGAMUSHI FEVER ON THE CHORIOALLANTOIC MEMBRANE OF THE DEVELOPING CHICK EMBRYO \*

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The rickettsiae of tsutsugamushi fever (*Rickettsia orientalis*) can be cultivated in the yolk sac of the developing chick embryo in the same manner as the other rickettsiae (Lewthwaite and O'Connor, 1943). This has been confirmed by others as well as by me. It was of interest to test the growth potentialities of tsutsugamushi rickettsiae on other media, including cultivation on the chorioallantoic membrane of the developing chick embryo, especially since it had been shown that the rickettsiae of epidemic typhus could be grown on this membrane (Zia, 1934). In contrast with the sparse growth of epidemic rickettsiae, when the organisms of tsutsugamushi fever were inoculated onto the chorioallantoic membrane a large ulcerated and nodular lesion was produced which thus far has developed in essentially its original form throughout 26 successive transfers.<sup>†</sup> The magnitude of the response elicited in the host tissue and the presence of numerous rickettsiae in smears from the lesion prompted me to make a more detailed study of the development and histologic character of the nodules. The results, which are presented here, are of interest because of the remarkable proliferative response induced in the host tissue by the presence of tsutsugamushi rickettsiae.<sup>‡</sup>

## METHODS

The initial lesions were produced by inoculating peritoneal exudate from infected mice, obtained when the animals were moribund, onto the chorioallantoic membrane of 10-day chick embryos. The peritoneal exudates were obtained from mice that had been infected with two strains of tsutsugamushi fever which had been isolated recently in New Guinea.<sup>§</sup> These are known as the Kostival and Shope strains. Each

\* Received for publication, June 2, 1945.

<sup>†</sup> The use of the chorioallantoic membrane as a site for growing tsutsugamushi rickettsiae was mentioned by Lewthwaite and O'Connor (1943). In this report, they stated that it "had given poor results." Recently, I had the pleasure of seeing Dr. Lewthwaite, and he showed me photographs of chorioallantoic membranes which had been infected with scrub typhus. At that time he had made no histological study of the lesions and had not studied their specificity. However, the gross appearance of the lesions was essentially identical with what I had obtained.

<sup>‡</sup> This study was initiated at the suggestion of Colonel Harry Plotz, M.C., Chief of the Division of Virus and Rickettsial Diseases, Army Medical School, and member of the U.S.A. Typhus Commission.

<sup>§</sup> Strains were isolated by Captain E. J. Bell, Sn.C., of the U.S.A. Typhus Commission.

sample of peritoneal exudate was diluted 1:5 with physiological salt solution, and 0.2 cc. amounts were placed on each chorioallantoic membrane. The eggs were then incubated at 37.5° C. for 6 to 7 days. The initial lesions which resulted were removed with sterile technic. Smears from them were stained by Giemsa's method, differentiated in acetone, and examined for rickettsiae. Subsequent egg passages were made by grinding a single lesion in 2 cc. of salt solution and inoculating 0.2 cc. of the suspension on each chorioallantoic membrane. Both strains of tsutsugamushi gave similar lesions.

For histological study, the lesion was clipped from the egg along with a wide area of chorioallantoic membrane and pinned out with glass needles on a layer of wax in a Petri dish. The preparation was then covered with Zenker's solution and fixed for 12 hours. After washing in water, the lesion was trimmed, dehydrated in alcohol, embedded in paraffin, sectioned at 4  $\mu$ , and stained by Wolbach's modification of the Giemsa method.

#### GROSS STRUCTURE OF THE LESION

The chorioallantoic lesions have varied in size from a pin-point thickening to a convoluted tumor-like mass which measured 18 by 11 by 5 mm. The average-sized lesion was 10 by 6 by 3 mm., as shown in Figures 1 and 2. The size of the lesion was influenced by the type and concentration of the inoculum.

At the height of its development (5 to 7 days), the lesion appeared as a large, white, nodular mass. On its external surface (where the inoculum was originally placed) there was typically a yellowish gray ulcer forming a sort of shallow concavity in the flat surface of the membrane (Fig. 1). In some cases this concavity was filled with a droplet of clear, viscid fluid.

The main body of the lesion was best seen by removing the chorioallantoic membrane and turning it over so that the lesion could be seen from the internal surface (Fig. 2). Typically, it consisted of a large, white, thickened nodule or cluster of nodules. However, in rare cases there might be "daughter colonies" in addition to the main nodular mass. Beneath the main body of the lesion was a stringy network of grayish precipitate which was enmeshed in the nodular mass but easily separable from it and which floated partially free in the allantoic fluid.

The nodular mass was well vascularized (Figs. 1 and 2). Whether this occurred through an original localization of rickettsial growth at the juncture of two blood vessels was not known, but it appeared that there must have been a certain amount of subsequent enlarging or diverting of blood vessels to supply the lesion, for many more large

vessels were found there than would be found in a similar area of normal membrane. In some cases, the nodular mass was so well vascularized that it was surrounded by large vessels, radially arranged like the spokes in a wheel.

Smears were made from the exudate above the ulcer, the nodular mass, the gray precipitate beneath the lesion, the allantoic fluid, the embryo, and the yolk sac. Rickettsiae were very numerous in the nodular mass, and sparse in the exudate above the ulcer, but were not found in any of the other places which were examined.

In order to determine the order of infectivity of the chorioallantoic lesion, serial dilutions of a suspension prepared from one lesion were made in the concentrations 1:5, 1:50, and 1:500, and inoculated in 0.2 cc. amounts onto the chorioallantoic membranes of three series of eggs. Typical large, thickened lesions were found in the lowest dilution used, like those obtained in routine passages. With a dilution of 1:50, the lesion was somewhat smaller (diameter of approximately 2 mm.), there was less thickening, and in place of the ulcerated surface there was a central hemorrhagic area. At the highest dilution, 1:500, there was a very small but definite lesion consisting of a whitish, opaque spot (0.5 to 1.0 mm. in diameter) surrounded by a transparent area. Grossly, there did not appear to be any appreciable thickening of the membrane. The lesion resembled the occasional "daughter colonies" which occurred in conjunction with a main nodular mass on membranes that were heavily infected.

When nodules were ground up and titrated in mice by intraperitoneal inoculation, infectivity was shown to be as high as  $10^{-7}$ . The disease induced in the mouse was identical to that produced by passage of other infectious materials.

#### HISTOLOGIC STRUCTURE OF THE LESION

Embryologically, the chorioallantoic membrane is derived from a fusion of the chorion with the allantoic sac, each of which consists of an epithelial layer of cells and a layer of mesoderm. The two membranes are fused so that the mesodermal layers are in apposition. Thus, in section, the chorioallantoic membrane consists of an outer layer of ectoderm (from the chorion), a middle layer of mesenchyme (from both chorion and allantois), and an inner layer of endoderm lining the allantoic sac. Normally, the thickness of the chorioallantoic membrane does not exceed 0.25 mm.

By far the most striking feature of the lesion produced by *R. orientalis* was the tremendous proliferative response evoked in the host tissue. The nodular mass might be 5 mm. thick, or as much as twenty



times the thickness of the normal membrane. Histologically, it was seen that the thickening was due primarily to a proliferation of the mesenchyme (Figs. 3, 4, and 5) and infiltration by ameboid "round cells." The prominent nucleoli and chromatin pattern in these "round cells" indicated that they were probably stem cells (*i.e.*, embryonic leukocytes) (Fig. 8). The outer, ulcerated surface of the lesion was bordered by rounded, partially necrotic cells instead of the normal epithelial layer. Immediately underlying this ulcerated edge was a layer of denser, more deeply staining mesenchymal cells resembling fibroblasts. Within the remainder of the nodular mass, which consisted of an enormously thickened layer of mesenchyme containing many blood vessels, there were centers of necrosis surrounded by a layer of mesenchymal cells of the fibroblastic type (just as underlay the ulcerated surface of the lesion) (Figs. 3, 4, 5, 8, and 9). Large numbers of ameboid stem cells infiltrated the mesenchyme and were aggregated around the necrotic areas (Fig. 8). Eosinophilic leukocytes were also very numerous in the nodular mass, particularly in the lumina of blood vessels. Although typical thrombi were not noted, the aggregation of stem cells and eosinophilic leukocytes often formed a tight mass which completely filled the lumen of the vessel. There was some inflammation of the blood vessel walls, especially within the areas of proliferation and necrosis. The endothelial cells were swollen and increased in number, and the wall of the vessel was often necrotic. The human lesion rarely contains eosinophilic leukocytes, and while phlebitis also occurs, it is not associated with necrosis.\* Because of these differences, it appears inadvisable to make a comparison between the chorioallantoic nodular mass and the human eschar.

The development of individual nodules within the chorioallantoic lesion appeared to depend on a local proliferation of the mesenchyme. Each center of proliferation, with its fibroblastic type of mesenchyme, enlarged and spread, and might merge with other centers of proliferation (Figs. 3 and 4). During this stage the nodular mass was formed and became increasingly vascularized. Eventually the cells in the center of each proliferative mass became necrotic (Fig. 5). In cases where the center of proliferation lay near the surface of the nodule, the necrotic mass might break through the epithelium and form a secondary ulcer (Figs. 4 and 5).

In favorable preparations, rickettsiae appeared to lie free in the interstices of the mesenchyme of the entire lesion (Figs. 11, 12, and 13). They were scarce in the centers of the necrotic areas, probably because

\* Personal communication from Captain A. C. Allen, M.C., of the Army Institute of Pathology.

of poor conditions for growth, but were particularly abundant around their edges. The stem cells which collected around the centers of infection seemed to be actively engaged in ingesting rickettsiae, for they had short, broad pseudopods, and many were filled with rickettsiae. The rickettsiae themselves, which lay free in the tissue, had the typical diplobacillary form with bipolar granules (Fig. 11). However, after they had been ingested by stem cells they appeared shorter and more densely stained. Both the outer and inner epithelial layers of the chorioallantoic membrane were infected with rickettsiae in the region of the nodular mass (Fig. 10). The organisms were more intensely stained when inside epithelial cells, and were more numerous in the outer layer (on the surface of which the original inoculum was placed) than in the inner (endodermal) epithelium.

In spite of the large numbers of rickettsiae which they contained, the epithelial layers were ordinarily only slightly thickened (Figs. 3, 4, and 5). In some cases, however, the epithelium had been so stimulated to proliferate that large convoluted or papillomatous masses were formed (Figs. 6 and 7).

When infected yolk sac was used as a source of inoculum on the chorioallantoic membrane, the size of the lesion and yield of rickettsiae were greatly increased (a 1:20 suspension by weight was made by grinding the sac and diluting with physiological salt solution; 0.2 cc. of this inoculum was placed on each chorioallantoic membrane). The nodular mass, though larger, did not differ structurally from the smaller type produced when infectious material was obtained from mouse peritoneal exudate.

#### SPECIFICITY OF THE LESION

The character and magnitude of the lesion and presence of rickettsiae in it would indicate that it was directly caused by these organisms. However, the chorioallantoic membrane is a particularly good site for growth not only for bacteria but also for fragments of tissue, so that it appeared desirable to test whether the irritation and subsequent nodular mass were produced specifically by the rickettsiae. The possibility that extraneous organisms might be involved was eliminated by making blood agar cultures of the suspensions used for each chorioallantoic passage, and by testing the nodules for infectivity in mice. Lesions were taken from the 5th and 21st passages, ground in salt solution, and injected intraperitoneally into four mice each (1:5 dilution). Material from the 5th passage caused death of all mice in 6 to 9 days; those which received inoculum from the 21st passage died in 5 days. The animals showed typical symptoms of tsutsugamushi disease with many

intracellular and extracellular rickettsiae in the peritoneal exudate. No organisms, other than the rickettsiae, were demonstrable either in the blood agar cultures or in the peritoneal exudates from mice which were infected with nodular material.

The chance that fragments of tissue in the inocula might grow and form chorioallantoic grafts did not appear probable when the source of infectious material was peritoneal exudate from adult mice. When the inocula were obtained from embryonic tissues, however, such as infected yolk sac or chorioallantoic nodules, it seemed very probable that fragments of embryonic tissue might become established as grafts and form proliferative nodules. This possibility was tested by preparing suspensions from normal yolk sac and chorioallantoic membrane in the same way as was done with infected tissues, and inoculating them onto chorioallantoic membrane for the same (7 day) growth period. Sporadic, pin-point colonies of tissue were found on some membranes, but these were infrequent, insignificantly small, and entirely different in appearance from the nodules produced by tsutsugamushi rickettsiae. Furthermore, when tissues infected with tsutsugamushi rickettsiae were repeatedly frozen and thawed so as to destroy all living cells, the inoculum prepared from such frozen tissues produced typical, large, ulcerous nodules on the chorioallantoic membrane. It seemed clear, therefore, that the lesion was not due to the presence of living tissue cells in the inoculum.

A further possibility is that the proliferative nodule may be caused only secondarily by the rickettsiae. The organisms might multiply in the chorioallantoic tissues, causing destruction of cells, and the necrosis *per se* might be sufficient stimulus to form a nodule. Or the rickettsiae might be only one of a number of stimuli which could cause irritation and sufficient tissue reaction to form a nodule. To test whether necrotic tissue was involved, fragments of embryonic tissues (yolk sac and chorioallantoic membrane) which had been killed by freezing, were placed on the chorioallantoic membrane. After 7 days of incubation, the membranes were examined for lesions. In most cases (10 of 11) there was a yellowish white, slightly thickened area where the dead tissue was in contact with the chorioallantoic membrane, but in no case was there a formation of nodules or extensive vascularization such as was obtained when rickettsiae were present. These experiments suggest, therefore, that dead tissue is in itself not a sufficient stimulus for the formation of nodules.

To test the possibility that tsutsugamushi rickettsiae might be only one of a number of stimuli which might lead to the same result, *viz.*, nodule formation, I inoculated different kinds of rickettsiae on the

chorioallantoic membrane. A grayish thickened area was produced on the membrane by fièvre boutonneuse rickettsiae. Large numbers of organisms were seen in smears from this area, and the embryos died of the infection in 3 to 4 days. The rickettsiae of Rocky Mountain spotted fever did not kill the embryos until 4 to 6 days after inoculation. Progressively larger thickenings and nodules were formed, depending on the length of the growth period, until by the sixth day the nodular mass was comparable in size to that produced by tsutsugamushi rickettsiae. However, an ulcerated outer surface was obtained only with *R. orientalis*. Zia (1934) has demonstrated that the rickettsiae of epidemic and Mexican typhus can produce occasional small nodules, although the usual reaction was a simple thickening of the chorioallantoic membrane. His brief description of the nodules suggests that they are similar in structure, although much less extensive, than those I have obtained by using rickettsiae of tsutsugamushi and Rocky Mountain spotted fever. It appears, therefore, that nodule formation, *per se*, is not a specific phenomenon, but may be produced by several kinds of rickettsiae.

Other evidence is available which indicates that the chorioallantoic membrane may respond to many diverse stimuli by producing nodules. Danchakoff (1916) found that when adult chicken spleen (and some other tissues) was grafted on the chorioallantoic membrane, the hematopoietic organs of the host embryo became enlarged. The most apparent enlargement was in the host spleen, and was caused primarily by an intense proliferation of both the mesenchyme and the young stem cells. Centers of necrosis appeared in the spleen as a result of this excessive accumulation of tissue with an inadequate capillary supply. Similar results were obtained by Willier (1924) when small pieces of adult chicken thyroid gland, liver, or thymus were grafted on the chorioallantoic membrane. Large nodular grafts were produced which contained necrotic areas. Histologically, it was found that the grafts were formed in large part by proliferation of the mesenchyme and a tremendous infiltration of leukocytes. In each case, the spleen of the host embryo was correspondingly enlarged and showed a number of necrotic areas in section (Willier, 1924).

I have examined the spleens of infected embryos which had large chorioallantoic nodules produced by the growth of tsutsugamushi rickettsiae, and have found that they, too, are several times normal size. There was greater enlargement of the host spleen when the chorioallantoic nodules were larger. Smears from the host spleen contained large numbers of stem cells and many rickettsiae. Danchakoff (1916) postulated that certain kinds of grafted tissue liberate sub-

stances which stimulate the hematopoietic organs and induce intense proliferation of their cells. Reasoning by analogy, it appears probable that rickettsiae liberate toxins which incite a similar activity of the hematopoietic system, with a mobilization of stem cells at the centers of infection. The resultant chorioallantoic nodule (and enlarged spleen) thus would represent a secondary and nonspecific effect of the growth of rickettsiae.

### SUMMARY

1. A large nodular mass of tissue with an ulcerous outer surface is formed by the growth of *Rickettsia orientalis* on the chorioallantoic membrane of the chick embryo. The nodule, which is proliferative in nature and highly vascularized, usually becomes 8 to 10 mm. in diameter and 4 to 6 mm. thick after 7 days of growth.

2. The nodular mass is formed by extensive proliferation of the mesenchyme around centers of rickettsial growth. As each locus of rickettsial growth increases in size, the centrally located cells become necrotic. Embryonic leukocytes—"stem cells"—migrate into the nodule and ingest rickettsiae from the fringe of growth surrounding the necrotic areas.

3. Rickettsiae are found scattered throughout the mesenchyme of the nodular mass, but are particularly abundant around the necrotic areas. They also occur in large numbers in the epithelial layers.

4. A feature of the lesion is the remarkable response of the host tissue to the growth of rickettsiae. There is not only a tremendous proliferation of mesenchyme and the formation of layers of primitive, fibroblast-like cells around loci of rickettsial growth, but in some cases the epithelium also is stimulated to form multi-branched and thickened, papillomatous growths. The formation of nodules on the chorioallantoic membrane is not considered specific for tsutsugamushi rickettsiae. Grossly similar nodules are produced by the growth of rickettsiae of Rocky Mountain spotted fever on this membrane. The size of such nodules is apparently directly correlated with the length of the growth period.

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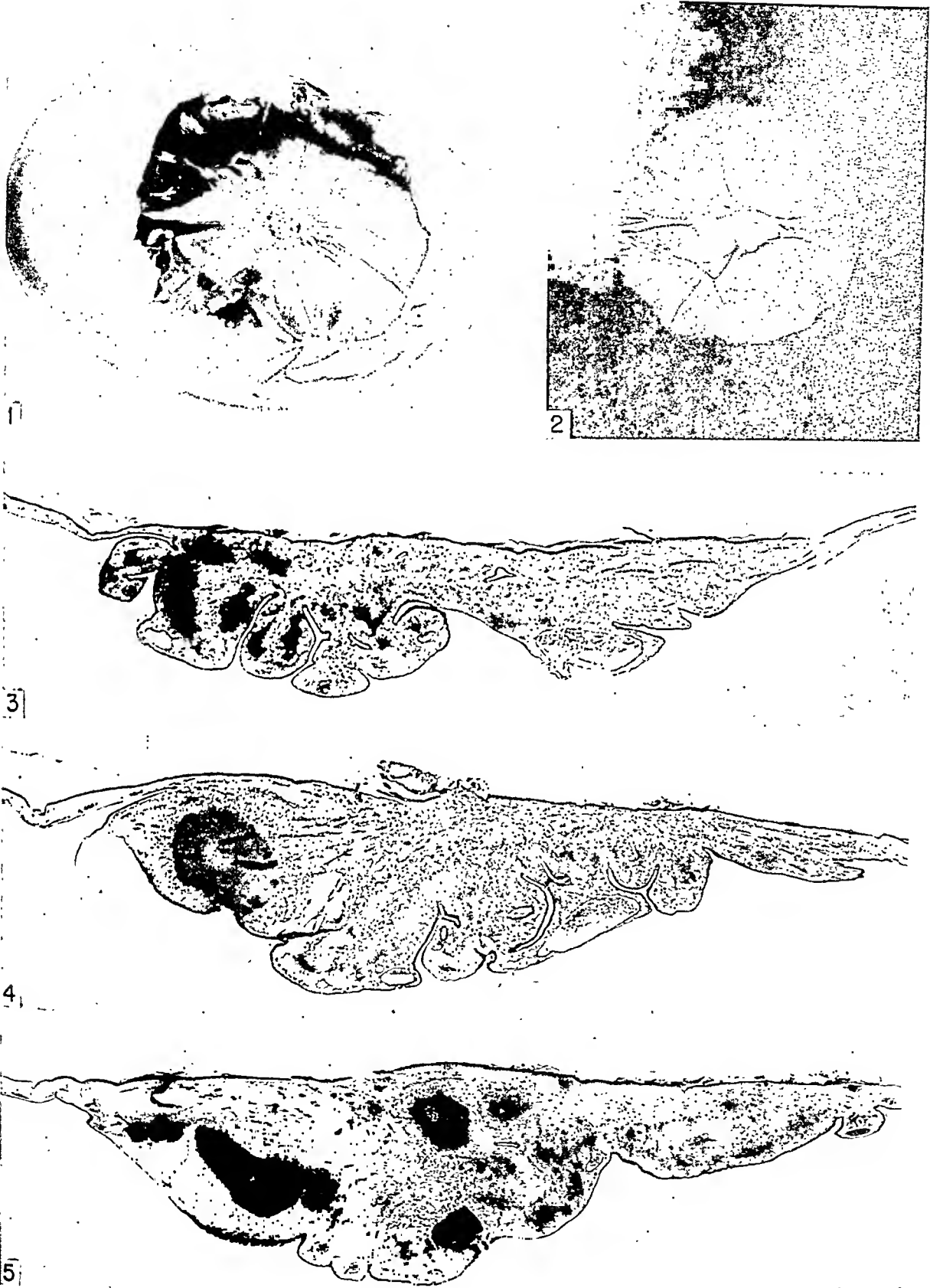
[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

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### PLATE 20

- FIG. 1. *In situ* view of the lesion produced by *tsutsugamushi rickettsiae* on the chorioallantoic membrane, 12th passage. Of note is the central ulcer which, in this case, is filled with a clear, viscid fluid.  $\times 1$ .
- FIG. 2. A reverse view (from the inner surface of the allantoic sac) of the lesion in Figure 1, showing the nodular mass. Large blood vessels supply the mass, and punctate "daughter colonies" are seen apart from the main nodule.  $\times 1$ .
- FIGS. 3-5. Sections through chorioallantoic nodules. Figure 3 shows an early stage, with centers of proliferation indicated by islands of darkly staining mesenchyme cells. In Figure 4 the centers of proliferation have spread and started to become confluent. At the left of the section is a large necrotic mass which has broken through the allantoic endoderm. The outer (chorionic) epithelium has started to slough off in the center of the section where the primary ulcer will form. Figure 5 shows advanced necrosis. A fringe of proliferating cells surrounds each necrotic area. At the lower part of the section, one necrotic mass has formed a channel into the allantoic sac.  $\times 8$ .



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Rickettsiae of Tsutsugamushi Fever



PLATE 21

FIGS. 6 and 7. Portions of the allantoic endoderm, showing papillomatous-like thickening and multi-branched proliferation of the epithelium.  $\times 120$ .

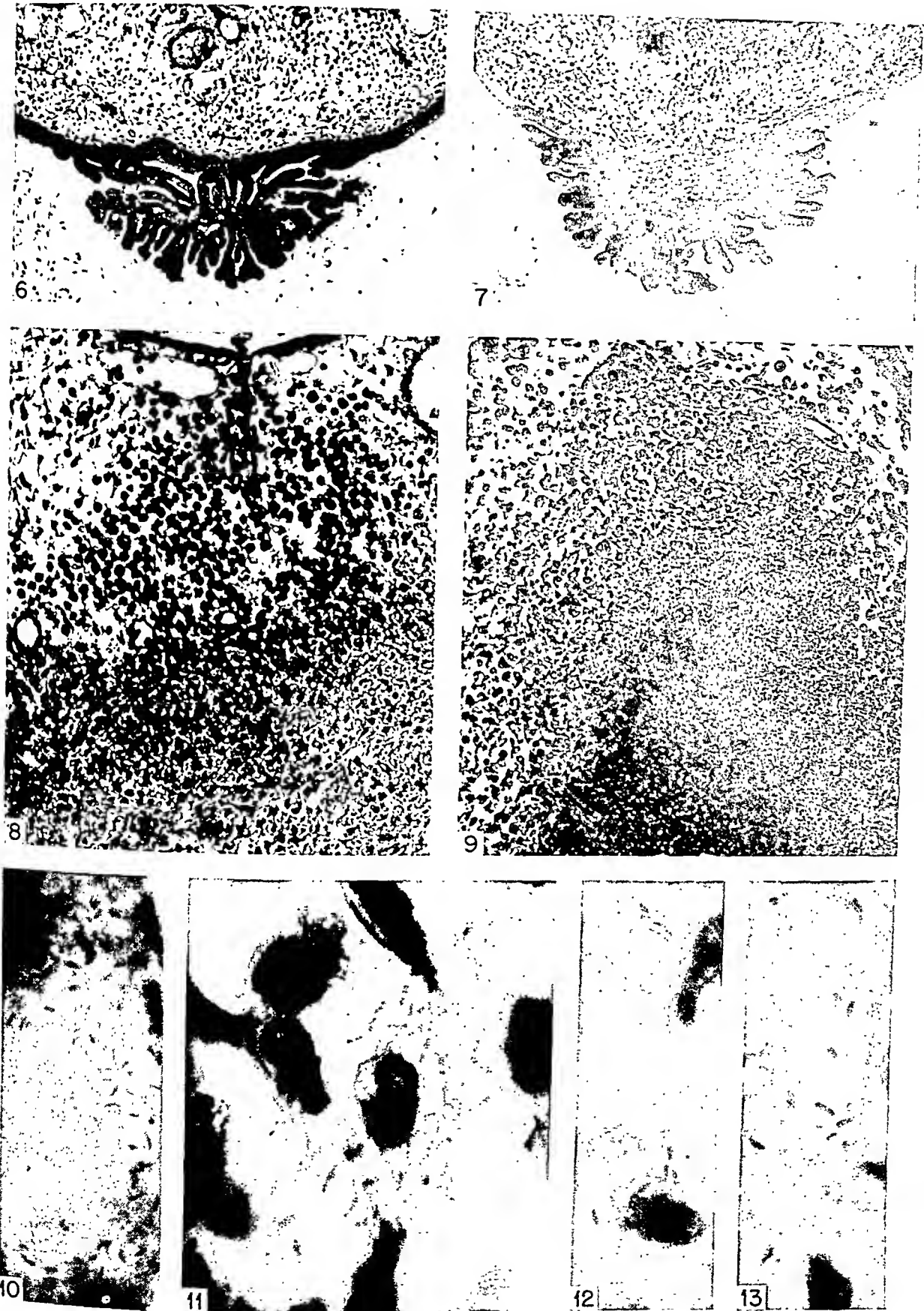
FIG. 8. A portion of the peripheral zone of a center of proliferation, showing the large number of "stem cells" which migrate into the mesenchyme and ingest rickettsiae.  $\times 240$ .

FIG. 9. Portion of a necrotic area, showing the surrounding ring of deeply staining mesenchyme in which the cells resemble fibroblasts, and in which rickettsiae are numerous.  $\times 240$ .

FIG. 10. Surface view of the chorionic epithelium, showing many rickettsiae.  $\times 1660$ .

FIGS. 11-13. Rickettsiae in the mesenchyme of the nodular mass. Figure 11 is from the fringe of an area of proliferation. Of note are the typical diplobacillary forms.  $\times 1660$ .

Figures 3-13 are from U. S. Army Medical Museum negatives, numbers: 78866, 78868, 78867, 80420, 80419, 79150, 79151, 78924, 78870, 78869, and 78865, respectively.



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Rickettsiae of Tsutsugamushi Fever



# LESIONS OF SKELETAL MUSCLES IN RHEUMATOID ARTHRITIS NODULAR POLYMYOSITIS \*

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Multiple nodular inflammatory lesions in peripheral nerves in cases of rheumatoid arthritis were reported for the first time in previous papers.<sup>1,2</sup> They were found in 3 of 5 cases. Identical findings observed in 5 more cases of this disease are to be reported in another paper. Since muscular atrophy, occasionally of rapidly progressive nature, is seen clinically in many cases of rheumatoid arthritis, an attempt was made to search for anatomopathological changes to which muscular atrophy could be related.

## MATERIAL AND TECHNIC

Muscle in quantity is not easily obtainable during life. The patient is often reluctant to permit the removal of tissue for biopsy for scientific purposes alone without prospect of therapeutic benefit. Pieces of muscles taken for biopsy are usually small, rarely over 2 gm., and are taken at random from a large muscle of the arm or leg. In 4 cases of rheumatoid arthritis such specimens were taken from the gastrocnemius muscle; in 1 of these an additional specimen was taken from the deltoid muscle. In 2 cases specimens from the triceps muscle were taken for biopsy. In another case of rheumatoid arthritis a mid-thigh amputation of both legs was performed, because of immobility and marked deformity of the patient's lower extremities. The peripheral nerves of both legs were dissected. Some muscle tissue, adherent to these nerves, was obtained incidentally. In 2 cases pieces of muscle were taken at autopsy, in 1 from the pectoral muscle, in the second from the rectus abdominis, pectoral and iliopsoas muscles. The latter case was found at autopsy among our control material. Altogether, muscles from 9 cases of rheumatoid arthritis were examined.

Since pathological involvement of skeletal muscles in cases of systemic disease has been described frequently, a large number of controls was desirable. Up to the present time, 196 control cases, selected from routine autopsies and surgical operations without any previous knowledge of the history or diagnosis, have been examined. There were

\* Aided by grants from The Children's Fund of Michigan and from the National Foundation of Rochester, Michigan.

Received for publication, March 12, 1945.

specimens from the recti abdominis, the pectorales, the iliopsoas, the gluteal muscles, the bicepses and tricepses.

The specimens of muscle were fixed in formalin, and blocks were cut transversely and longitudinally; after embedding in paraffin, sections were made  $7\ \mu$  thick. Hematoxylin and eosin, cresyl violet, van Gieson's, and reticulin (Wilder) stains were used. Frozen sections could not be made because the small amount of muscle tissue obtained had to be embedded entirely in paraffin. Likewise there was no material available for fixation in alcohol, which excluded staining for glycogen. For similar reasons histopathological studies of striation and sarcomeres requiring special technics were not made.

No descriptions of similar findings are to be found in medical literature. The only description of pathological lesions resembling ours is in a paper by Curtis and Pollard.<sup>3</sup> They found increase in interstitial nuclei of the muscle fibers, small perivascular infiltrations, and atrophy of muscle fibers in specimens taken for biopsy from the calf muscles of 12 patients with rheumatoid arthritis. The purpose of their paper was to determine whether Felty's syndrome was a clinical entity or just an "expected complex of rheumatoid arthritis."

## REPORT OF CASES

### *Case 1*

The patient (Eloise Hospital) was a white female, 54 years old, the mother of 11 children. For 19 years she had had rheumatoid arthritis involving nearly every joint. Roentgenograms revealed bony ankylosis of the left knee joint. The joint space of the right knee was lost on the lateral half, and on both sides there was some loss of joint space between the humerus and the ulna with partial bony ankylosis. The muscles of the lower extremities were markedly atrophic.

### Morphological Description

The specimen was taken from the middle portion of the left gastrocnemius muscle and was 1.6 cm. long and 0.9 cm. thick in its largest transverse diameter. Grossly, it did not differ from a piece of normal muscle.

The pathological findings had to be separated into those which were inflammatory and those which were degenerative. At least five inflammatory lesions were found in each section. They were entirely separated from one another and consisted of nodular accumulations of lymphocytes and plasma cells. The plasma cells were more frequent in the peripheral portions of the nodules. The shapes of the nodules varied markedly. There were triangular, pyramidal, and spindle-shaped nodules (Fig. 1) located in the perimysium and the endomysium, separating single muscle fibers from each other. Occasionally

a small amount of adipose tissue was seen at one or both ends of the nodule (Fig. 2). In addition to the nodular accumulations, there were a few well demarcated foci composed of a smaller number of lymphocytes. There was no necrosis in the lymphocytic nodules, but occasionally there were seen a few epithelioid cells that had arisen from the cells of capillary endothelial linings or from the sarcolemma. Van Gieson's stain revealed an increase in collagenous connective tissue fibers while the reticulin stain failed to reveal an increase in reticulin fibers in the nodules.

The degenerative lesions of muscle fibers were well defined and were very irregularly distributed in close proximity to normal muscle fibers. A single degenerated fiber was never seen isolated between normal fibers; always three or more degenerated fibers were together. The degeneration of muscle fibers was marked by an increase of sarcolemmal nuclei, closely packed together; swelling or, more often, extreme shrinkage of fibers; a change of the staining reaction of the fibers from a bright red to pale red or pink (Fig. 2), and an increased coarseness in the cross striations with larger but regular interspaces between the striations. Occasionally a slightly swollen muscle fiber showed a transition to shrinkage, indicating a segmental degeneration. Extremely shrunken muscle fibers in cross section were not much larger, or were even smaller, than human red blood cells (Fig. 3). The nucleus of such a shrunken muscle fiber was located at the periphery. Occasionally an empty space like a vacuole was seen as the core of the shrunken muscle fiber, leaving at the periphery a pale, ring-shaped, membrane-like structure in which the nucleus was embedded. The nuclei were small, spindle-shaped or curved, and contained dark-staining chromatin. Occasionally a cross section through a shrunken muscle fiber revealed 8 to 15 small, dark nuclei in the center of the fiber.

In the walls of capillaries and larger blood vessels and inside perimysial nerve fiber bundles, as well as in neuromuscular spindles, no inflammatory nodules were seen. However, in perimysial locations adjacent to intramuscular nerve fiber bundles and to the adventitia of blood vessels nodular lymphocytic infiltrations were found (Figs. 4 and 5).

### *Case 2*

The patient (Eloise Hospital), a white female, 48 years old, had had progressive rheumatoid arthritis for 19 years. She showed flexion deformities of the elbows, wrists, and fingers; complete ankylosis of the right wrist and partial ankylosis of the left; and nearly complete ankylosis of the left ankle and both knees with subluxation. Roentgenograms revealed a marked loss of joint space in the right interphalangeal joints, loss of joint space in the right elbow, and ankylosis of the right knee. The skin over the legs and on the face was glossy. There was severe muscular atrophy.

### Morphological Description

The specimen for biopsy was taken from the middle portion of the left gastrocnemius muscle. It measured 2.4 cm. in length and was 0.6 cm. in its largest transverse diameter. There was no grossly visible change.

The microscopical findings again had to be separated into inflammatory and degenerative changes. The inflammatory lesions appeared in separated and remote groups. They were well circumscribed and occupied the perimysium and the endomysium of muscle bundles. Their shape was nodular but more irregular than that of the previously reported<sup>1,2</sup> perineuritic nodules; there were often small nests of cells connected with the nodule that in tongue-like fashion infiltrated farther into the endomysium between two muscle fibers.

The inflammatory cells were lymphocytes and plasma cells, the former being distributed very regularly throughout the nodules, the latter found in the periphery of the nodular accumulations. There was no definite inner zone of necrosis or of epithelioid cells. The nodules contained capillaries and increased collagenous connective tissue while the reticulin fiber network was scant.

Muscular degeneration was seen in very irregular distribution. Well preserved muscle fibers were seen in close proximity to severely atrophic muscle fibers (Fig. 6). There was swelling as well as shrinkage of the fibers, the swollen fibers showing discoloration, and usually a darker color. Cross striations were coarser and the interspaces larger. Instead of straight outer contours, the swollen muscle fibers showed wavy or irregularly distorted outlines. The shrunken muscle fibers were occasionally paler; nevertheless, the cross striations were often distinctly seen. The reduction in size was more than two-thirds of normal; in crosscut fibers the size of an individual fiber was often smaller than that of a red blood cell. The nuclei in these shrunken fibers were peripherally located; however, there was an occasional accumulation of 8 to 10 nuclei closely packed together. In some of the markedly shrunken muscle fibers only a nucleus and a membrane-like outer structure remained, and the inner core was represented by a vacuole. The perimysial connective tissue was increased in amount and showed more nuclei than are normally present. There were accumulations of nuclei even in the muscle fibers that did not exhibit degenerative changes. Occasionally an increased amount of adipose tissue could be seen between muscle fibers.

In the walls of capillaries and larger blood vessels and inside of perimysial nerve fiber bundles as well as in neuromuscular spindles no inflammatory nodules were seen. However, in the vicinity of two small

perimysial nerve fiber bundles, nodular infiltrations involving the perineurium and adjacent interstitial tissues were seen (Fig. 7).

### Case 3

The patient (Harper Hospital) was a white male, 53 years of age, who was admitted to the hospital on May 29, 1944, complaining of painful and swollen joints. The condition had followed the removal of a tumor of the bladder 4 years previously. The joints were involved in the following order: feet, knees, hands, elbows, neck, and shoulders. Over a period of several months the joints had become swollen, stiff, and sore. The extremities were moist and cool. The condition was worse during cold or wet weather. The patient had become gradually weaker, and he noted that his muscles wasted as time went on. On two occasions he had had transitory "lumps" at his elbows. His muscles were always sore, especially those of his arms and legs. He had received fever therapy and a course of gold injections at another hospital, but he felt that his condition had not been improved.

The patient was a large man, somewhat pale, with marked deformity of all joints. There was fusiform swelling of the phalanges. The metacarpophalangeal and the carpometacarpal joints showed diffuse periarticular swelling; there was marked interosseous atrophy. The elbow joints could not be fully extended, and the arms could be raised only to an angle of  $45^{\circ}$  from his body. Motion of the feet and knees was limited. No nodules were felt. The heart was not enlarged, and no murmurs were present. The blood pressure was 140/85 mm. Hg. All reflexes were hyperactive. Sensation was normal everywhere except for some hyperesthesia of the feet.

During his stay at the hospital the patient had prostigmine, methylsalicylate, myochrysine, blood transfusions, massive doses of sodium salicylate, high vitamin-D medication, and physiotherapy. There was moderate improvement.

*Laboratory Findings.* Examination of the blood showed: Hemoglobin, 86 per cent; red blood cells, 4,460,000; white blood cells, 8,400, with a normal differential count. Several determinations of the sedimentation rate gave a maximum of 27 mm. and minimum of 21 mm. in 60 minutes. Blood nonprotein nitrogen was 36.6 mg.; blood calcium, 11.5 mg.; and blood phosphorus, 2.1 mg. per cent. A Kahn test of the blood was negative.

*Radiological Examination.* Soft tissue swelling was seen about the majority of the proximal interphalangeal joints with decrease in the joint spacing of all carpal bones and a generalized demineralization of all bones examined. No hypertrophic changes could be seen. There was roentgenographic evidence of arthritis, of a rheumatoid type, involving the left hand. Studies made of the right knee revealed thinning of the articular cartilages with considerable effusion into the bursae.

### Morphological Description

Specimens were taken from the left gastrocnemius and deltoid muscles. The two pieces of tissue from the gastrocnemius muscle were 1.1 by 0.9 cm., and 0.4 by 0.2 cm. The piece from the deltoid muscle was approximately 1 by 1 cm. Grossly, there were no visible changes.

Except for an increase in sarcolemmal nuclei, no degenerative or inflammatory lesion was found in the gastrocnemius muscle. The specimen from the deltoid muscle showed two small inflammatory nodules of typical appearance, without atrophic lesions of the muscle fibers.



The inflammatory cells again were lymphocytes and a few plasma cells. Rarely, polymorphonuclear cells were seen.

In the walls of capillaries and larger blood vessels, in perimysial nerve fiber bundles, and in neuromuscular spindles no inflammatory nodules were seen.

#### *Case 4*

The patient (Harper Hospital) was a white male, 46 years old, whose chief complaint was painful, stiff, swollen joints. Six years before he had noticed occasional pain in the muscles of his left arm. This slowly progressed to the other arm, and then the knees became involved. He received treatment in many forms during this period of time, the last being 100,000 units of vitamin D daily. On this he improved slightly. At the time of entrance to the hospital he was totally disabled and could not walk. Occasionally, when the joint condition grew worse, hoarseness developed.

Past illnesses were septic sore throat in 1932; gonorrhea in 1935; malaria in 1935; and pneumonia in 1940. There was nothing remarkable in the family history, and there was nothing of importance in the physical examination except for the findings which concerned the joints. The heart was not enlarged. There were no murmurs and no arrhythmia. Blood pressure was 147/75 mm. Hg. The spleen was palpable. Examination of the joints revealed ulnar deviation with inability to extend the metacarpophalangeal joints. The elbows were slightly flexed as were the feet and knees.

*Laboratory Findings.* Examination of the blood showed: Hemoglobin, 80 per cent; red blood cells, 4,700,000; white blood cells, 8,750, with 60 per cent segmented forms, 34 per cent lymphocytes, and 6 per cent band forms. The sedimentation rate was 23 mm. in 60 minutes. Chemical studies showed nonprotein nitrogen of the blood to be 36.6 mg.; calcium, 11.1 mg., and sugar, 80 mg. per cent. A Kahn test upon the blood was negative.

*Radiological Examination.* Roentgenograms were made of the chest, teeth, paranasal sinuses, and gallbladder; no abnormal findings were recorded. Roentgenograms of the hands showed marked narrowing of the joint spaces with considerable demineralization of the bone, and there was some periarticular soft tissue swelling. The cartilaginous disks had almost entirely disappeared.

*Clinical Diagnosis.* The case was diagnosed as typical rheumatoid arthritis. The patient was discharged to his private physician with recommendations for the resumption of gold therapy, of which he had had inadequate amounts several years before.

#### Morphological Description

The specimen from the left triceps consisted of two pieces, one of oval shape measuring 0.5 and 0.6 cm., and a very small piece composed of but ten muscle fibers longitudinally cut. There was no grossly visible change in either of them. However, sections stained by hematoxylin and eosin showed grossly visible, dark blue dots separated from each other. The size and shape of these dots varied from round to elliptic. With a hand lens seven of these areas were counted in the larger pieces and two in the smaller. Their shape was irregular due to elongated processes of dark blue color extending from some of them.

In serial cuts these dark blue-staining spots extended through 10 to 15 consecutive sections. Since each section was  $7\ \mu$  thick, the average size of such a nodule was 0.070 to 0.105 mm. in thickness, the maximum length being about 1 mm.

Microscopically, the inflammatory lesions were well circumscribed, although there were processes from the nodular mass infiltrating the perimysium and the endomysium (Fig. 8). These elongated processes were composed of the same cells as the nodules. The inflammatory cells were lymphocytes, many of them immature with vesicular nuclei. There were also rather abundant plasma cells in a more peripheral location, and mast cells outside the nodules. In larger nodules circumscribed accumulations of the epithelioid cells with nuclei poor in chromatin and definite nucleoli were seen (Figs. 9 and 10). These nuclei seemed to be related to capillary endothelial cells or sarcolemmal cells. They were grouped together, usually nearer to the periphery of the nodule. In sections stained with van Gieson's stain, the increase of collagenous fibers was marked, and the location of the inflammatory process in endomysium and perimysium could be ascertained. Many of the larger nodules showed a somewhat loose structure with spaces surrounding the lymphocytes and plasma cells. No stroma was recognized in these spaces; a reticulin stain revealed scanty reticulin fibrils. In one elongated nodule the accumulation of inflammatory cells was interrupted by a crosscut capillary, the walls of which were not infiltrated by inflammatory cells. Aside from larger nodules, small foci of lymphocytic infiltration were seen more widely spread.

Of special interest were small inflammatory foci where a muscle fiber was surrounded by endomysially located inflammatory cells, lymphocytes, and plasma cells. Here a beginning degenerative alteration of the muscle fiber was seen (Fig. 11). There was an occasional peculiar arrangement of small vacuoles in longitudinal rows parallel with the longitudinal striations of the fiber, which then did not show any cross striation (Fig. 12). These muscle fibers were much paler than the normal ones in their vicinity and toward their peripheral portions showed increased large nuclei poor in chromatin. These nuclei regularly contained two nucleoli, were elliptic or ovoid, and showed a distinctly outlined nuclear membrane; occasionally the nuclei were distributed in rows close together at the periphery of the muscle fiber (Figs. 11 and 13). Rarely, one large elongated nuclear mass containing dark-staining chromatin was seen instead of the numerous pale nuclei described before (Fig. 14). In the pale gray-staining sarcoplasm of these degenerating muscle fibers peculiarly pale-staining granular

dots of minimal size were seen. Coarser cross striation, beginning longitudinal fibrillation, and beginning vacuolization around hyperplastic muscular nuclei were seen in some of the muscle fibers (Figs. 15 and 16). Most of the muscle fibers, however, did not show beginning or advanced degeneration although there was occasional swelling.

The epimysium showed no signs of inflammation, nor did the walls of capillaries and of larger blood vessels, the perineurium of intramuscular nerves, or the neuromuscular spindles.

### *Case 5*

The patient, a white male, 61 years old, was admitted (Harper Hospital) on August 4, 1944, because of pain in his joints. He had suffered from rheumatoid arthritis for 4 months, being troubled with pain that seemed to originate at the back of his neck. His attending physician believed that the discomfort on flexing his neck was due to "abscessed" teeth. These were removed. Following the extraction, however, the pain spread to the joints of his upper extremities, affecting chiefly the elbows and fingers. During the month prior to his admission he developed pain in both knees.

There was nothing remarkable on physical examination except a slight increase in the transverse diameter of the heart; a blowing systolic murmur was heard at the apex and transmitted toward the base,  $P_2$  being slightly accentuated. Blood pressure was 120/60 mm. Hg. The margin of the liver was palpable; the spleen was not palpable. Except for hyperactivity in his reflexes there were no abnormal neurological findings. There was marked pain on flexion of the knee joints and slight muscular atrophy above and below the joints. In the upper extremities there was slight fusiform swelling of the first phalangeal joints and some thickening of the distal joints of his fingers. The amount of pain and stiffness was variable; at one time he was unable to lift his arm to his head. There were slight elevations of temperature during his stay in the hospital.

The patient was placed on myochrysine therapy with marked improvement.

*Laboratory Findings.* Examination of the blood showed: Hemoglobin, 76 per cent; red blood cells, 3,910,000; white blood cells, 9,050, with a differential count of 6 per cent band forms, 68 per cent segmented forms, 24 per cent lymphocytes, and 2 per cent eosinophils. Sedimentation rate was 20 mm. in 60 minutes. Non-protein nitrogen of the blood was 48.6 mg. per cent. The Kahn test was negative. There was a slight trace of albumin in the urine and occasional fine granular casts.

*Radiological Examination.* Studies of both hands revealed some periarticular swelling of soft tissues with a very slight demineralization of the bones. Although there was no disturbance in joint spacing except slight thinning of the cartilages of the joints, particularly of the metacarpophalangeal joints, the film study showed arthritis of an early atrophic type.

### Morphological Description

Two pieces were taken from the left gastrocnemius muscle. One was 0.9 and 0.4 cm. in longitudinal and transverse diameters respectively; the other was 0.7 by 0.5 cm. Grossly, there was no visible change. Microscopically, there were again lesions of two distinct types, inflammatory and degenerative. The inflammatory lesions were of a

definitely nodular type. In the entire section five nodules were seen, four in the perimysium and endomysium, and one in the epimysium. The nodules seen in crosscut muscle fiber bundles were endomysial; the muscle fibers themselves were not invaded by inflammatory cells (Fig. 17). As before, the inflammatory cells were lymphocytes and plasma cells. The nodules were small, much smaller than in the previous case; they did not contain epithelioid cells and apparently represented earlier stages of muscle involvement than in the previous case. Nevertheless, the collagenous connective tissue was increased in and around the nodules. The epimysial nodule had developed between blood vessels, but there was no involvement of the vascular walls. The inflammatory cells were spread between the adventitial layers of the blood vessels, and no inflammation was present in the adventitia, media, or intima of the vascular walls.

In spite of the smallness of the inflammatory nodules, scanty but definite signs of degeneration of muscle fibers were seen. The degenerating muscle fibers were located between normal muscle fibers, the ratio of normal to degenerating muscle fibers being 20 to 1. There was a definite color change of the degenerating fibers; some exhibited a more bluish tinge, others a bright, peculiarly refractile, yellowish color (in hematoxylin and eosin stain). The discolored fibers, when longitudinally cut, showed a marked longitudinal fibrillation. The degenerated fibers showed somewhat irregular lines of cross striations and coarser interspaces between the transverse striae. Most of the bluish muscle fibers showed an increase of large, pale nuclei with one or two large nucleoli; the nuclei were sometimes arranged in longitudinal rows. In these fibers vacuolization at the periphery of the muscle fiber or around the large nuclei was occasionally seen (Fig. 18). Again, this early lesion seemed to be segmental, occupying a short longitudinal portion of a single fiber. Neuromuscular spindles were free of inflammation.

### *Case 6*

The patient, a white female, 29 years old, was admitted (Harper Hospital) on August 14, 1944, having been in good health until 13 months previously when she had begun to suffer with rheumatoid arthritis. Three weeks after the birth of her first child she had felt pains and stiffness in the muscles and joints. At the outset, the symptoms came and went without any evidence of swelling, but a short time later she noticed slight swelling, redness, and tenderness. At that time an "abscessed" tooth was extracted. There was no history of any other previous acute infection, or chronic illness. The patient had been carefully examined in two other medical clinics: in one no diagnosis was made; in the other, because of an increased sedimentation rate, she was told that the possibility of rheumatoid arthritis had to be considered. Roentgenograms of the joints at each clinic revealed no changes suggestive of rheumatoid arthritis.

At the first examination the patient complained as much of pain in the muscles, particularly in the deltoid muscles, as of pain in the joints. She was worse at night, and when awakening found it difficult to move because of pain and stiffness.

The skin was somewhat glossy, delicate, moist, and smooth. The heart sounds were clear and of good quality. There was a slight spindle-shaped swelling of the fingers of each hand, and a very slight interosseous atrophy of the dorsum of each hand. The right great toe was stiff, but no enlargement could be determined. The reflexes of the extensor muscles of the forearm, the biceps, and the patellars were hyperactive. No clonus was elicited. During the 9 days that the patient was in the hospital her temperature rose to 101° F. on two occasions and promptly subsided. The pulse rate varied from 80 to 110. Myochrysine therapy was begun during hospitalization.

The patient presented the clinical features of early rheumatoid arthritis.

*Laboratory Findings.* Examination of the blood gave the following results: Hemoglobin, 78 per cent; red blood cells, 3,910,000; white blood cells, 4,150, with a normal differential count. The sedimentation rate was 24 mm. in 60 minutes. A Kahn test upon the blood was negative. Examination of the urine gave only negative findings.

*Radiological Examination.* Examined in dorsopalmar projections, the hands showed no evidence of abnormality. When these roentgenograms were examined 1 month later, one roentgenologist thought that some slight narrowing of the joint spaces was present, but no destructive process was discernible.

### Morphological Description

Two small pieces were obtained for biopsy from the left triceps muscle. The larger was 0.4 cm. in longitudinal and transverse diameters; the smaller was a pyramidal-shaped piece with a 0.3 cm. longitudinal axis and a 0.4 cm. base. Microscopically the inflammatory nodules were smaller than those seen in the three previous cases; their morphological appearance, however, was identical. Individual degenerating muscle fibers were seen of the same type as those in case 5, but they were fewer.

### Case 7

The patient (Eloise Hospital) was a white female, 27 years of age, who was admitted in January, 1942, because of swollen and painful joints. At the age of 11 she had had swelling and redness of the left knee that disappeared after rest in bed; the pain did not migrate. The patient did not recall that she had had any fever. She had been in good health until about 6 years prior to admission, when pain and stiffness appeared in the right knee, both shoulders, and in the right temporomandibular joint. Later she had swelling and severe pain in the knees. An impacted wisdom tooth was extracted, the right knee joint was aspirated, and she received vaccine and physiotherapy, but no relief was forthcoming. She was able to get about on crutches. Following the delivery of a full-term child 3 years before admission to the hospital, her condition became worse. Swelling and tenderness extended to other joints of her body, notably to the fingers of both hands. Two years before admission both legs and the left elbow joint were put in casts. No improvement followed attempts to straighten the legs and to manipulate the joints.

On admission, the skin was pale, cool, thin, and shiny, especially over the extremities. The heart was not enlarged nor were there any murmurs. All joints

of the body were involved, with marked deformity, limitation of motion, and fixation of some of them, especially the knees and ankles.

*Laboratory Findings.* The blood showed: Hemoglobin, 10 gm.; red blood cells, 4,200,000; white blood cells, 4,400, with 40 per cent filamented forms, 4 per cent nonfilamented forms, 39 per cent lymphocytes, 6 per cent large mononuclear cells, and 11 per cent eosinophils. The sedimentation rate was 20 mm. in 60 minutes. A Kahn test was negative. Urinary examination showed no abnormality, and the electrocardiographic tracings were considered normal.

*Radiological Examination.* The fingers and wrists showed osteoporosis and sharply outlined joint margins. Similar changes were seen in both elbows, knees, and feet. The right knee showed, in addition, a fused patella and apparent fusion of the tibia and fibula. In both ankles there was tibial-tarsal fusion. The proximal phalanges of both feet were dislocated; marked hallux valgus was present.

*Clinical Diagnosis and Course.* The diagnosis was advanced rheumatoid arthritis, trophoneurotic disturbances, and hypochromic microcytic anemia.

Treatment consisted of blood transfusions, sedatives, and physiotherapy. Kirschner wires were inserted into both tibiae and traction applied. Two months later osteotomy of the right knee was performed and a plaster dressing at 10° flexion applied. One month later roentgenograms showed fusion of the tibia and femur, and union of patella with the right femur. The ankylosis and deformity of the lower extremities gave the patient constant pain and almost total immobility. She was able to use her upper extremities somewhat. The patient and her husband demanded consideration of the fact that she could be more easily handled at home if the useless legs were removed. First the right leg was removed at the mid-thigh, and 3 weeks later the left leg at the mid-thigh. She made an uneventful recovery and was discharged.

At the time of amputation our interest was focused on the investigation of peripheral nerves. By chance, some muscle tissue adherent to the left tibial nerve and another piece of muscle adherent to the left superficial peroneal nerve were removed. The nerves showed typical nodulous rheumathritic perineuritis.

### Morphological Description

In the muscle tissue adherent to the left tibial nerve two nodules were seen. One was the largest in this series of cases. It was seen grossly in stained sections as a deep-blue dot, 1 mm. in transverse diameter and 1.1 mm. in longitudinal axis. The other nodule was small and was seen only microscopically. The muscle tissues in the vicinity of the left superficial peroneal nerve showed three small nodules.

Again, inflammatory and degenerative lesions were distinguishable. The large nodule showed a massive accumulation of lymphocytes and plasma cells, the latter at the periphery. Epithelioid cells were not conspicuous; there were, however, remnants of degenerating muscle fibers in the center of the nodule as well as at its periphery (Figs. 19 and 20). Not far from the nodule, in the perimysium, an artery was seen with nodular inflammation of its wall, mostly in the media and adventitia (Figs. 21 and 22). A marked increase in collagenous connective tissue fibers was seen in the nodule and in its surroundings, with no increase in reticulin fibers. In the vicinity of the nodule the muscle fibers showed severe degeneration, a lack of cross striation, vacuolization, dis-

tortion of the straight longitudinal form with wave-like outer contours, and an increase in large nuclei that were poor in chromatin and contained one or two prominent nucleoli. Occasionally the muscle fibers were broken up into smaller, irregular fragments containing these large nuclei.

In the muscle in the vicinity of the left superficial peroneal nerve one perineurial nodular accumulation of lymphocytes and plasma cells was seen; the perineurium of a small intramuscular nerve fiber bundle was completely infiltrated with lymphocytes and plasma cells (Fig. 23). The other two nodules were endomysial without relationship to nerves or blood vessels. There was a severe shrinkage of muscle fibers in isolated nests as described in previous cases.

### Case 8

The patient was a white male, 55 years old. When he entered Eloise Hospital in August, 1941, complaining of painful, swollen joints, he had been suffering with rheumatoid arthritis for more than 11 years. Eleven years before he had noted pain in the left hand and elbow and some swelling and reddening of the joints. Progressively, over a period of several months, the shoulders, knees, hands, and feet became involved. Treatment was of no avail, and gradually his hands and feet became deformed. Numbness and tingling of the toes occurred at various times, and cramping of the calf muscles was an occasional symptom. Some shortness of breath was experienced on exertion. For the past 3 years slight swelling of the ankles had been noticed.

On admission his temperature was 98° F.; the pulse, 90; and respiration, 20. The eyegrounds showed some arterial tortuosity; the tonsils were atrophic; and the mouth was edentulous. Signs of fluid were present in the left pleural cavity, and a few moist râles were present at each base. The heart dulness merged with the dulness at the left base. There were no murmurs and no arrhythmia, although the aortic second sound was slightly accentuated. Blood pressure was 158/90 mm. Hg. All joints of the body were involved. There was marked limitation of motion of the larger joints. The fingers were flexed and rigid. Atrophy of all muscles of the body was apparent, especially those of the hands, arms, legs, and thighs. The feet and legs were cool, the right cooler than the left. Pulsation in the dorsalis pedis was less noticeable in the right foot than in the left. The reflexes were all markedly exaggerated. Clonus, muscle spasm, and fibrillation were not present. Subcutaneous nodules were present at each elbow. A nodule was excised for biopsy.

In September, 1941, cardiac decompensation developed, but the patient recovered temporarily following appropriate therapy. In August, 1942, the right great toe became infected, and gangrene of the foot followed. Cardiac decompensation returned. A right mid-thigh amputation was performed but the patient's condition did not improve and he died of cardiac failure on October 10, 1942, 6 weeks following amputation.

*Laboratory Findings.* Examination of the blood showed: Hemoglobin, 9.5 gm.; red blood cells, 3,780,000; white blood cells, 9,600; blood sedimentation on admission, 20 mm. in 60 minutes. A Kahn test was negative. Examination of the urine showed albuminuria of mild degree, with a few hyaline and finely granular casts; the average specific gravity was 1.020. Electrocardiograms revealed severe myocardial damage.

*Radiological Examination.* There was marked contracture deformity of the left hand. All proximal phalanges were dislocated in relation to the heads of the metacarpals. The joints of the wrists were narrowed. The joint spaces of the left elbow were narrowed and the margins sclerotic and irregular. Both patellae were fixed and the joint spaces of the knees were narrowed with sclerotic margins. The same changes were present in the ankles.

*Clinical Diagnosis.* Advanced rheumatoid arthritis, subcutaneous nodules, generalized arteriosclerosis, arteriosclerotic heart disease, myocardial failure, bilateral hydrothorax, gangrene of the leg, and peripheral arteriosclerotic disease.

### Morphological Description

The findings at autopsy were bilateral purulent empyema, bilateral chronic adhesive pleuritis, hypertensive and atherosclerotic heart disease, chronic fibrinous pericarditis with obliteration of the pericardial sac, atherosclerosis, and arteriolar sclerosis of both kidneys.

At the autopsy, three pieces of tissue were taken from the left pectoralis major muscle. In each of the three there was nodular lymphocytic inflammation, with usually three to five nodules in each section. They were located in the perimysium, more rarely in the endomysium. Small, elongated, spindle-shaped, circumscribed, inflammatory foci were seen in the endomysium (Fig. 24). An increase of collagenous connective tissue fibers and a lack of reticulin fibril production were apparent as usual. The perimysial nodules were often seen in the vicinity of blood vessels periadventitially, but never adventitially (Fig. 25). There was an occasional nodule in the perineurium of an intramuscular nerve bundle. The muscle fibers showed irregularly distributed swelling and a loss of cross striation. There were a few severely shrunken muscle fibers, usually in bundles of two or three between normal fibers. The shrinkage was seen in both crosscut and longitudinally cut fibers.

In one of the pieces of muscle tissues there was arteriolar sclerosis with atherosclerosis in the larger arteries.

### Case 9

This case was found among our 196 control cases. For controls, specimens were removed routinely at autopsy from the pectoral, iliopsoas, and rectus abdominis muscles. They were fixed in formalin and brought to the laboratory without any clinical or post-mortem data.

The patient was a white male, 55 years of age, who had died in the admitting room of the hospital after 1 week of illness. No other data were available. At autopsy left lobar pneumonia (pneumococcus, type VII), fibrinous pleuritis, hyaline perisplenitis, and perihepatitis were found. In addition the patient showed ulnar deviation of both hands and bilateral hallux valgus with lateral deviation of the other toes of both feet. He had multiple subcutaneous nodules; one at the knee joint was saved for microscopical examination. The diagnosis of arthritis had been made post mortem.



### Morphological Description

Pieces from the iliopsoas and pectoralis muscles showed no conspicuous lesions; however, in the piece from the rectus muscle two distinct inflammatory nodules were found. They were located in the perimysial connective tissue near a nerve and an arteriole. There was no infiltration of the endoneurium of this nerve and no perivascular arrangement. The inflammatory cells were lymphocytes and plasma cells; van Gieson's and reticulin stains revealed the same characteristics seen in the nodules in the muscles of the previously described cases. Definite degenerative changes of the muscle fibers were not found.

The subcutaneous nodule was composed of a large central zone of necrosis in which cells and connective tissue had completely disappeared, being replaced by necrotic structureless masses. Peripheral to the necrotic zone, rows of radially arranged proliferating cells of fibroblastic origin were seen. The necrotic center was sharply demarcated by the palisading arrangement of these fibroblasts. In the peripheral zones of the nodule marked infiltration with lymphocytes and plasma cells was present, usually in perivascular arrangement. There were three smaller nodules of miliary size in the vicinity of the large nodule. In these smaller nodules the central necrotic zone was in the beginning stage, showing numerous fibroblasts resembling epithelioid cells, and both fine and thick, bright-staining, collagenous connective tissue bundles in a somewhat radiating arrangement.

### DISCUSSION

The muscular lesions reported herein were of an inflammatory nature. There were also degenerative changes in muscle fibers. The inflammation was of a nodular type, lymphocytes and plasma cells were abundant, mast cells occasional, and polymorphonuclear cells and eosinophils rare or absent. The size of the nodules varied from those seen grossly in stained sections to very small ones. The shape was round or oval, triangular or spindle-shaped, often elongated, with processes of inflammatory cells infiltrating the endomysium between two or more muscle fibers. There were also smaller foci composed of twenty or less lymphocytes. The inflammatory nodules were located in the endomysium and the perimysium, rarely in the epimysium. In the larger nodules cells of an epithelioid type were seen, usually not in the center of the nodule but at the periphery. The origin of these cells could not be established definitely. They were derived either from capillary endothelial cells or from muscular nuclei, for there was a

definite resemblance between these nuclei and those of degenerating muscle fibers. The inflammatory nodules were found in each of the nine cases of rheumatoid arthritis examined. Striking features of the nodules were an increase in bundles of collagenous connective tissue fibers and a lack of reticulin network. Even in larger nodules a loose structure was evident with spaces around the inflammatory lymphocytes and plasma cells.

There were various stages of degeneration of muscle fibers. In late cases of rheumatoid arthritis, such as our cases 1 and 2, both of 19 years' duration, the degenerative changes were advanced and were represented by a marked atrophy, fatty metamorphosis, and severe distortion of the outer contours of the fibers. The early stages of muscle degeneration were seen in our cases 4, 5, and 6 (of 6 years', of 4 months', and of 13 months' duration respectively). They were distinct from the late stages, in which shrinkage was the most prominent feature. In the early stages of involvement there was a change in color to a bluish tinge or, more rarely, to a peculiar bright yellow. The cross striations were not straight but somewhat wavy, and the interspaces between them were coarser than normally. There were pale muscle fibers and peculiar vacuolization of these fibers. The bluish discoloration was somewhat similar to that reported in the basophilic granular degeneration of muscle fibers in trichinosis. The muscular nuclei were more numerous, and an alteration of position and shape was seen. Instead of being just beneath the sarcolemmal sheath the nuclei were located more toward the interior of the fibers or in the central longitudinal axis. The nuclear shape changed from rod-like to oval or round, and the nuclei became twice as large as normal. The perinuclear cytoplasm was not distinct; there was, however, perinuclear vacuolization. Densification of perinuclear sarcoplasm was seen also, the sarcoplasm exhibiting a darker color and a distinctly granular appearance. The nuclear chromatin was poor; the nucleolus was large, and there were often two nucleoli, which took the acid stain. Amitotic divisions and incomplete segmentation between nuclei were also found. Rarely an elongated giant nucleus was observed, its longitudinal axis being three times as long as the axis of one of the swollen nuclei. The proliferated nuclei appeared in longitudinal rows parallel with the longitudinal axis of the muscle fiber. Such rows contained as many as twenty nuclei.

In three cases the perineurium of intramuscular nerves showed nodular inflammation, and occasionally periadventitial and mural nodular inflammation of blood vessel walls was seen in the perimysium and the

epimysium. However, the early stages of the disease showed nodular inflammation in the endomysium and the perimysium without relationship to intramuscular nerve fiber bundles, blood vessels, or neuromuscular spindles.

### *Classification*

The anatomopathological lesion in the muscular tissues must be designated as nodular polymyositis with secondary muscular atrophy. The name, polymyositis, has been selected because of the multiplicity of the inflammatory lesions and their wide distribution over the entire skeletal muscular system (gastrocnemius, other unidentified muscles of legs (case 7), deltoid, triceps, pectoral, and rectus abdominis muscles). The term "nodular" has been chosen in spite of occasional, more diffuse, inflammatory changes in the vicinity of the nodules because in most of the sections the muscular nodules appear as well demarcated lesions.

Perimysium and endomysium as well as muscle fiber and nuclei are of mesodermal origin. Since connective tissue, in general, and perimysium and endomysium, in particular, have a low grade of differentiation, the histological response to various infections and to other stimuli is limited. Among the infectious diseases a number of characteristic histological reactions of connective tissue can be recognized, for instance: tuberculous caseous necrosis, gummatous reticular fibrosis, fibrinoid necrosis with cellular palisading in subcutaneous nodules of rheumatic fever and rheumatoid arthritis, and Aschoff bodies in the myocardium. All of these more or less specific granulomatous reactions are characterized by the appearance of circumscribed nodules of varying sizes, composed of special tissue elements in special arrangements. Hence these processes are distinguishable from diffuse inflammatory infiltrations. The character of the infiltrating inflammatory cells is another factor determining the classification of inflammatory changes. Granulomas, in general, exhibit the subacute or chronic picture of lymphocytic cellular infiltration. Besides the characteristic appearance in nodules, there is often a lymphocytic accumulation in ring-like fashion at the periphery of the lesion. This lymphocytic ring is typical in nodular perineuritis of rheumatoid arthritis; the inner zone is occupied by epithelioid cells or fibroblasts. In the small nodular granulomas in the muscles of our patients, lymphocytes and plasma cells were evenly distributed throughout the entire granuloma; epithelioid cells were seen only in the larger nodules. Lymphocytes and plasma cells predominated; eosinophils and polymorphonuclear cells were rare. In rheumathritic perineuritis the nerve fibers themselves did not ap-

pear to be damaged while in rheumatoid polymyositis a severe degeneration of muscle fibers accompanied the inflammatory process.

The muscular lesion found in our cases of rheumatoid arthritis may be classified as nodular polymyositis.

Nodulous rheumathritic perineuritis as reported in previous papers<sup>1,2</sup> and nodular polymyositis described herein are essential findings in rheumatoid arthritis. Both lesions are of an inflammatory nature, are found in perineurial, and in perimysial and endomysial locations, and are alike in their histological patterns. Therefore, the findings in peripheral nerves and muscles may be classified in a single phrase as nodular neuromyositis of rheumatoid arthritis.

### *Specificity of Muscular Lesions*

The histological observations reported herein are thought to be of specific character.

1. *Nodular Appearance and Distribution.* One of the main characteristics of the muscular lesions is their nodular appearance. Our material was not extensive enough to supply data on the distribution and preferred location of these nodular lesions in the entire mass of one skeletal muscle, to say nothing about the distribution in the entire skeletal muscular system or about the predilection of one or more individual muscles. Up to the present time the muscles examined are the gastrocnemius, deltoid, triceps, rectus abdominis, and pectoralis major. Our controls were the musculus pectoralis major, m. rectus, and m. iliopsoas. Our control material of 196 cases was not sufficient for a completely adequate comparison. Further, our control muscles showed other pathological lesions (atherosclerotic and arteriolar lesions, cartilaginous metaplasia in perimysial adipose tissue, post-traumatic abscess formation, trichinosis) in addition to those found in the cases of rheumatoid arthritis. Only in one control case were small lymphocytic infiltrations found in the endomysium and perimysium; these, however, were without secondary muscular atrophy. This was in a case of subacute bacterial endocarditis superimposed on old rheumatic heart disease. Another control case (reported herein as case 9) proved to be a case of rheumatoid arthritis.

Characteristic of the muscular change was the irregular distribution of the inflammatory lesions throughout the muscle tissues; in one section one to five nodules were seen, and the remaining perimysial spaces were without inflammatory lesions, or the lesions were very mild compared with the nodular accumulations. In serial sections these nodular inflammatory lesions did not extend through more than 10 to 15 sections, each 7  $\mu$  in thickness. Occasionally nodules were found in the

perineurium of small intramuscular nerves and in the walls of small arteries and veins. The intramural vascular inflammation was less often seen than the periadventitial arrangement. The neuromuscular spindles were never involved. An interesting histological detail was noted; namely, the increase of collagenous connective tissue and the decrease of reticulin fibers in the nodules.

2. *Constancy of Occurrence and Uniformity in Appearance. Differential Diagnosis.* Of a total of 10 muscle specimens from 9 cases, 7 were obtained for biopsy (2 from one case), and 3 through amputation of legs or at autopsy. Nine of these specimens showed definite and identical pathological lesions. The muscle tissues of each case were positive. In case 3 the specimen from the deltoid muscle was positive but not that from the gastrocnemius muscle. Although the amount of muscle tissue examined in each individual case was very small compared with the total quantity in the body, the high incidence of positive findings in various muscles is an indication of the wide distribution of nodular polymyositis throughout the skeletal muscular system in rheumatoid arthritis.\*

Identical findings of nodular polymyositis in all 9 cases suggest the specific nature of this lesion. The control muscles did not show any lesions comparable to those found in rheumatoid arthritis. The controls were muscles taken from routine autopsies (196 cases) and 7 cases in which pathological lesions in muscles could be expected; namely, 1 case each of congenital myatonia, of myasthenia gravis, of amyotrophic lateral sclerosis, of progressive muscular dystrophy, of dermatomyositis, of lupus erythematoses, and of trichinosis. In addition to the previously mentioned pathological findings in our control material from routine autopsies, muscular degeneration without inflammatory lesions was found in cases of amyotrophic lateral sclerosis, of congenital myatonia, and of progressive muscular dystrophy. The inflammatory lesions in dermatomyositis and trichinosis were easily distinguished from those seen in the muscles of cases of rheumatoid arthritis. In dermatomyositis the inflammatory changes were diffusely spread and did not occur in nodular fashion as they did in the muscles of rheumatoid arthritis. In trichinosis, inflammation was close to encysted trichinae, with a definite capsule and numerous eosinophilic cells. There are a number of other diseases with definite muscular involvement; for example, glanders and Chagas' disease, that could not be examined for lack of material. On the other hand, in at least 7 of

\* Five additional muscle specimens from 5 other cases of typical rheumatoid arthritis have shown identical lesions.

the 9 cases of rheumatoid arthritis from which muscle tissues were taken, there was not the slightest possibility of any other disease; they were typical cases of rheumatoid arthritis without any complication.

3. *Morphological Similarity to Other Lesions Seen in Rheumatoid Arthritis.* There is a striking resemblance between the muscular lesions in rheumatoid arthritis and those in peripheral nerves; the nodular arrangement is seen in muscles and peripheral nerves alike. Both lesions have the same type of cellular infiltration, lymphocytes and plasma cells. The location in the perimysium corresponds to that in the perineurium. The nodular perineurial inflammation in small intramuscular nerves is identical with that seen in nodulous rheumathritic perineuritis. The general pattern of the lesions found in the muscles is quite similar also to that of lesions in the synovia, in subcutaneous nodules, and in scleromalacia perforans. An inflammatory nodular character prevails everywhere. In one of our cases (case 9) multiple subcutaneous nodules were present, one of which was examined microscopically. It presented the typical histological picture of a subcutaneous nodule in rheumatoid arthritis.

4. *Histological Comparison with "Fibrositis."* In favor of the specificity of the muscular lesions in rheumatoid arthritis is the entirely different histological picture in "fibrositis," which occasionally is characterized by the appearance of gross fibrous nodules. Hench,<sup>4</sup> declaring the fibrositic nodules as the "signposts of the disease," emphasized the disappointing results following biopsy. He found little or no histological abnormality. Other observers (Collins,<sup>5</sup> Slocumb,<sup>6</sup> Buckley<sup>7</sup>) agreed that the structural changes in fibrositic lesions are meager. The pathological findings in "fibrositis" as first described by Stockman;<sup>8</sup> namely, numerous fibroblasts, serous or serofibrinous exudate, and thickening of walls of the small blood vessels and nerve sheaths, were considered by Collins as unimportant. Collins emphasized the absence of cellular infiltration as the only important fact of Stockman's investigation. At any rate, there is not the slightest similarity between the muscular lesions in rheumatoid arthritis and the findings in "fibrositis," a strong point favoring the specificity of polymyositis in rheumatoid arthritis.

5. *Comparison of the Skeletal Muscular Lesions in Rheumatoid Arthritis with Those in Rheumatic Fever.* Klinge<sup>9, 10</sup> showed photomicrographs of granulomas in the musculus constrictor pharyngis and in the diaphragm of a patient with a second attack of rheumatic fever and rheumatic endocarditis. He considered these rheumatic nodules in the striated muscles more or less characteristic. They were often found

in the vicinity of joints but were also independent of the joints, for example, in the diaphragm and the pharynx. Graeff<sup>11</sup> also found typical granulomas in the voluntary muscles in rheumatic fever.

An interesting finding, heretofore not reported, is the involvement of the blood vessel walls in the muscles of rheumatoid arthritis. Definite nodular arteritis and periarteritis could be seen in small muscular branches in some of our cases. Von Glahn and Pappenheimer<sup>12</sup> described specific lesions of the small peripheral arterioles and capillaries in 10 of 47 cases of rheumatic cardiac disease, but skeletal muscles were not included in their routine autopsy material. As Collins<sup>5</sup> stated, polyarteritis has been observed frequently in rheumatic fever, but in rheumatoid arthritis the vascular lesions seem to be confined to the joints or subcutaneous nodes. This statement is no longer valid.

### *Pathogenetic Relationship between the Various Local Manifestations in Rheumatoid Arthritis*

Rheumatoid arthritis is a systemic disease with many local manifestations such as inflammation in the synovia, in joint capsules, and in periarticular soft tissues; subcutaneous nodules; and inflammatory nodules in peripheral nerves and skeletal muscles.

1. *Relationship between Lesions of Joints, Periarticular Lesions, and Lesions in the Muscles.* In a previous paper,<sup>2</sup> emphasis was laid on the fact that the perineuritic nodules were not in contact with synovial or periarticular lesions. This independence applies also to the muscular changes, since specimens were taken for biopsy from gastrocnemius or triceps muscles far from any joint or periarticular tissues. In one of our cases the rectus abdominis showed nodular inflammation. There was no relationship of the muscular lesions to subcutaneous nodules, since, with two exceptions (cases 8 and 9), no subcutaneous nodules were present in the cases from which the specimens were taken.

2. *Relationship between Perineuritis and Polymyositis.* Theoretically it might be assumed that the muscular lesion results from damage done to the peripheral nerves in nodulous perineuritis. In our previous paper<sup>2</sup> reasons were presented why clinically manifested muscular atrophy seemed to us not at all related to perineuritic lesions. Also we were not able to disclose degenerative changes in axis cylinders or myelin sheaths of peripheral nerve fibers. Hence there is little foundation for the assumption that muscular lesions are secondary to perineuritis. Furthermore, the irregular distribution and the nodular type of lesion speak in favor of the autochthonous origin of the muscular lesions. There is often a perineurial inflammation of small intra-

muscular nerves, to be sure, but serial sections show this change to be discontinuous. The process certainly does not ascend from the intramuscular nerve twigs into the larger bundles of the nerve trunk. It also does not descend from a perineuritic lesion in the peripheral trunk into smaller intramuscular ramifications of nerve fiber bundles. There is no pathogenetic interdependence between the lesions in the trunks of peripheral nerves and the muscular lesions.

3. *Relationship between Polymyositis and Muscle Degeneration.* While perineuritis did not produce grossly or microscopically visible damage in adjacent nerve fibers, there seemed to be a close relationship between polymyositis and muscular degeneration. The myositis is certainly not a consequence of a primary muscle degeneration, for, in our control material of purely degenerative diseases affecting the skeletal muscular system, marked inflammatory changes were absent. In degenerative lesions of the muscle Hassin<sup>13</sup> has observed scanty infiltrations in the perimysium, but these are not nodular as they are in polymyositis of rheumatoid arthritis, and they are accompanied neither by perineuritis of the small intramuscular nerve fiber bundles nor by arteritis of intramuscular arterioles. In our cases of rheumatoid arthritis *early* stages of muscular degeneration were seen only when there was a severe endomysial inflammation; without inflammation no beginning muscle fiber degeneration was found. Thus, muscular degeneration in rheumatoid arthritis does not give rise to inflammation; rather, muscular degeneration is a consequence of the inflammatory nodular lesions because of the close spatial relationship of damaged muscle fibers to inflammatory foci. The irregular distribution of degenerated muscle fibers in close relationship to inflammatory lesions, the appearance of debris of muscle fibers in the nodules, and the absorptive phenomena of muscular tissue in intimate connection with the nodular inflammation are proof that muscular degeneration and atrophy are a consequence of the nodular inflammatory polymyositic process.

It may be asked why a similar secondary degeneration of the nerve fibers in peripheral nerve bundles was not found accompanying the perineuritic nodular inflammation in our previously reported cases. Endomysium, perimysium, and muscle fibers are all of mesodermal origin. Consequently an inflammatory process encroaching on the muscle fibers by way of the perimysium and endomysium does not encounter such a complete barrier as it does in the perineurium at the border between mesodermal and ectodermal tissues of the peripheral nerves. As a result of this barrier endoneurial inflammatory lesions were not seen or were extremely rare while endomysial inflammation was com-



mon. With perineuritic inflammation no degenerated nerve fibers or endoneurial inflammation could be seen, while endomysial and perimysial nodular inflammation with damage to the muscle fibers was prominent in the same section. The occurrence of both types of inflammation in the same section indicates that the inflammatory processes are identical and that the resistance of nerve fibers is due to histological and physiological dynamics by which the nerve fiber is much better protected than the muscle fiber.

In summary, the inflammatory lesions in the synovia, joints, and capsular and pericapsular tissues; the subcutaneous nodules; the nodular lesions in peripheral nerve trunks; and the nodular polymyositic inflammation with arteritis and perineuritis in skeletal muscles are concomitant lesions due to the same unknown cause but entirely independent of one another. They are coordinate, not subordinate. However, muscular degeneration is a consequence of the inflammatory lesion in the perimysium and endomysium.

#### *Clinical Significance of Nodular Polymyositis in Rheumatoid Arthritis*

Clinically, rheumatoid arthritis has to be treated as a chronic inflammatory process, for the similar character of the muscular lesions and the lesions in other structures (synovia, subcutaneous tissue, and perineurium) is strong evidence of a generalized inflammatory process, possibly of infectious origin. Since the infectious agent is unknown, only general principles of treatment can be applied. That gold (myochrysin) treatment is not entirely satisfactory is evident from the fact that muscle specimens taken from cases 3, 5, and 6, thus treated, revealed inflammatory nodules identical with those seen in our cases in which no gold treatment had been given recently or in which inadequate amounts of myochrysin had been administered several years before (case 4).

Polymyositic nodules are specific for rheumatoid arthritis. In the future the clinical diagnosis in early or doubtful cases of rheumatoid arthritis may be supported by biopsy of affected muscles. Further, it is possible that the value of therapeutic procedures may be controlled by such means.

The findings in rheumatoid arthritis are more closely related to those of rheumatic fever than to those of any other disease. Whether there is an etiological relationship between these two diseases cannot be established by morphological investigation. However, when both have been recognized in the same patient, rheumatic fever has always preceded rheumatoid arthritis.

Rheumatoid arthritis, even in its late stage, is not a disease that has terminated in scar formation but is a smoldering, active, distinctive entity as shown by the presence of inflammatory foci in the muscles and in the perineurium. The seemingly "burnt-out" clinical picture in old cases of rheumatoid arthritis without pains but with extreme stiffness in joints and with bony deformities is deceptive, since active inflammatory processes are found in the muscles and the peripheral nerves.

Stiffness and bony contractures are responsible for some limitation of muscular activity. However, we wish to emphasize our conclusion that the anatomopathological lesions herein described, rather than disuse, are responsible for the clinical picture of muscular atrophy in rheumatoid arthritis.

The multiplicity of the inflammatory lesions in the synovia and other articular and periarticular structures, in the peripheral nerves, and in subcutaneous tissues indicates a wide spread of the causal agent in cases of rheumatoid arthritis. With the recognition of nodular polymyositis a new link in this nosological chain has been found. Simultaneously, the primary inflammatory character of this disease is made more evident.

#### SUMMARY

1. The pathological findings reported in this paper comprise "nodular polymyositis." Together with previously reported perineuritis, it constitutes an essential lesion in rheumatoid arthritis, to be called nodular neuromyositis.
2. The muscular lesions are specific for rheumatoid arthritis, since they were seen in every one of the 9 cases of rheumatoid arthritis and since they were absent in control material from 196 routine autopsies of cases of other diseases and 7 selected cases in which pathological lesions in muscles could be expected.
3. The distribution of the lesions in small amounts of various muscles (gastrocnemius, other unidentified muscles of the legs (case 7), triceps, deltoid, pectoralis major, and rectus abdominis) taken at random, and the high incidence of the lesions in a minimal percentage of the entire skeletal muscular system indicate the wide spread of nodular polymyositis throughout the skeletal muscular system.
4. The muscular lesions fit into the general pattern of other lesions known in rheumatoid arthritis, such as those seen in the synovia and other tissues of the joints, in subcutaneous nodules, and in the perineurium of peripheral nerve trunks. They are all alike in their inflammatory and granulomatous nature. The muscular lesions differ from those seen in other diseases (myatonia congenita, muscle dystrophy,

myasthenia gravis, dermatomyositis, "fibrositis," trichinosis). Besides the endomysial location of the lesions, perivascular and perineuritic nodular arrangements are seen in the perimysium.

5. The muscular lesions are not related spatially to lesions of the synovia, the periarticular tissues, the subcutaneous nodules, or of the peripheral nerves. They are concomitant but independent tissue reactions to the same unknown agent. Our material is not complete enough to establish a chronological order for these various manifestations. However, it seems evident to us that the muscular involvement begins early, perhaps at the same time that lesions of the joints become manifest (see case 5).

6. There is a definite pathogenetic relationship between nodular polymyositis and degeneration and atrophy of the muscle fibers. While inflammation can occur without degeneration of muscle fibers, early muscle fiber degeneration is always combined with inflammatory lesions in the endomysium of the same fibers. Hence an irregular distribution of degenerated muscle fibers is the consequence of irregular spread of nodular inflammation. The absorption phenomenon of degenerating muscle in intimate relation with the nodular inflammatory foci is further evidence of the primary nature of the inflammation and the secondary nature of muscle fiber degeneration.

7. Various stages of muscle fiber degeneration are recognizable from the early change in staining quality; through invasion of the contractile substance by peculiar nuclei and the appearance of vacuolization, irregular outer contour, and irregular cross striation; to extreme shrinkage of the muscle fibers. This sequence seems to be related to the duration of the disease or to the time of the involvement of the particular muscle examined.

8. From a clinical standpoint our pathological findings are important as evidence of the wide distribution of the inflammatory and probably infectious process throughout the body in rheumatoid arthritis. The finding of nodular polymyositis in cases treated with gold compounds leads to the conclusion that our present treatment is not sufficiently effective. The presence of polymyositic nodules in old clinically "burnt-out" cases is evidence of a permanent and active disease process. In the future, biopsy of muscle may be an aid in diagnosis, particularly in early cases, and in the control of chemotherapeutic action.

We wish to express our gratitude to Drs. Charley J. Smyth and S. E. Gould of Eloise Hospital, Eloise, Michigan, and to Dr. Plinn Morse of the Department of Pathology at Harper Hospital, Detroit, Michigan, for granting the use of material.

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[ Illustrations follow ]

## DESCRIPTION OF PLATES

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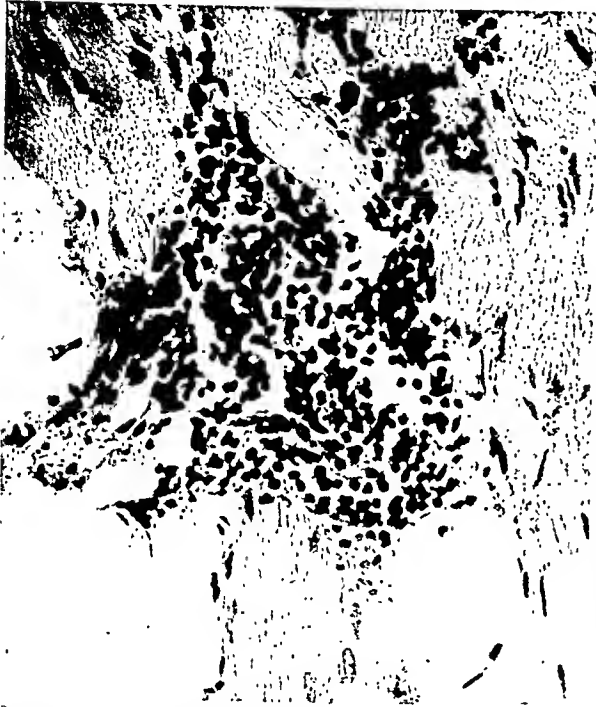
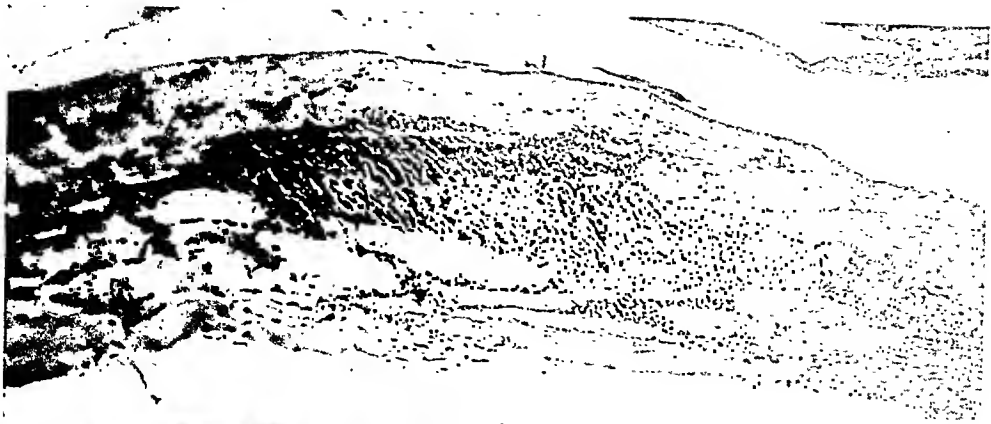
### PLATE 22

FIG. 1. Case 1. Specimen removed for biopsy from the gastrocnemius muscle. Spindle-shaped inflammatory nodule in endomysium. Hematoxylin and eosin stain.  $\times 100$ .

FIG. 2. Case 1. Specimen removed for biopsy from the gastrocnemius muscle. Smaller, triangular shaped, inflammatory nodule. Small amount of adipose tissue between two muscle fibers at the right lower angle of the nodule. At the upper end a pale muscle fiber is seen. Hematoxylin and eosin stain.  $\times 300$ .

FIG. 3. Case 1. Specimen removed for biopsy from the gastrocnemius muscle. General increase in sarcolemmal nuclei. Large muscle fibers, partially crosscut, are seen in the center of the field. Between them there are a number of extremely shrunken fibers. Hematoxylin and eosin stain.  $\times 100$ .

FIGS. 4 and 5. Case 1. Specimens removed for biopsy from the gastrocnemius muscle. Inflammatory nodules in a perimysial location adjacent to intramuscular nerves and to the adventitia of blood vessels. Hematoxylin and eosin stain.  $\times 100$ .



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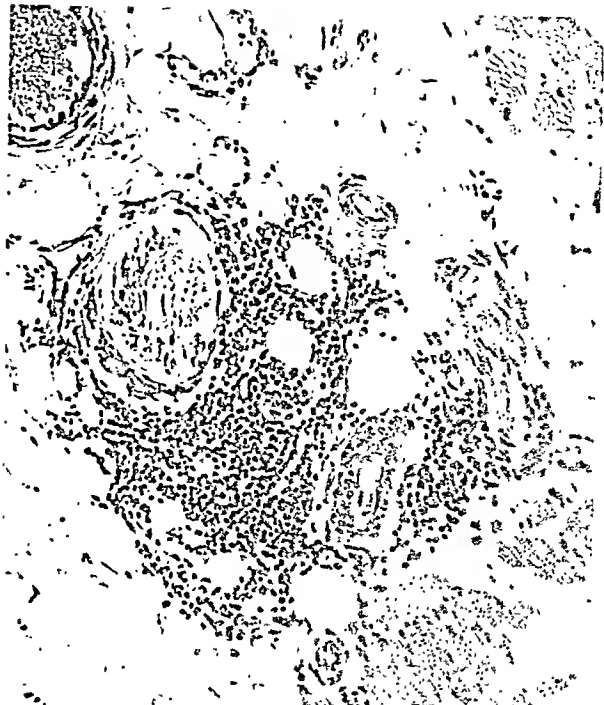
PLATE 23

- FIG. 6. Case 2. Specimen removed for biopsy from the gastrocnemius muscle. Longitudinally cut, markedly atrophic muscle fibers are shown to the right and below three swollen, discolored, and slightly vacuolated fibers. Hematoxylin and eosin stain.  $\times 150$ .
- FIG. 7. Case 2. Specimen removed for biopsy from the gastrocnemius muscle. Inflammatory nodule in perimysial location between two small intramuscular nerves. Hematoxylin and eosin stain.  $\times 150$ .
- FIG. 8. Case 4. Specimen removed for biopsy from the triceps muscle. Well circumscribed, endomysial, nodular inflammation with extensions of infiltrating cells from the body of the nodule. Hematoxylin and eosin stain.  $\times 150$ .
- FIG. 9. Case 4. Specimen for biopsy from the triceps muscle. A large inflammatory nodule with accumulations of epithelioid cells near its periphery. Cresyl violet stain.  $\times 150$ .

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Skeletal Muscles in Rheumatoid Arthritis

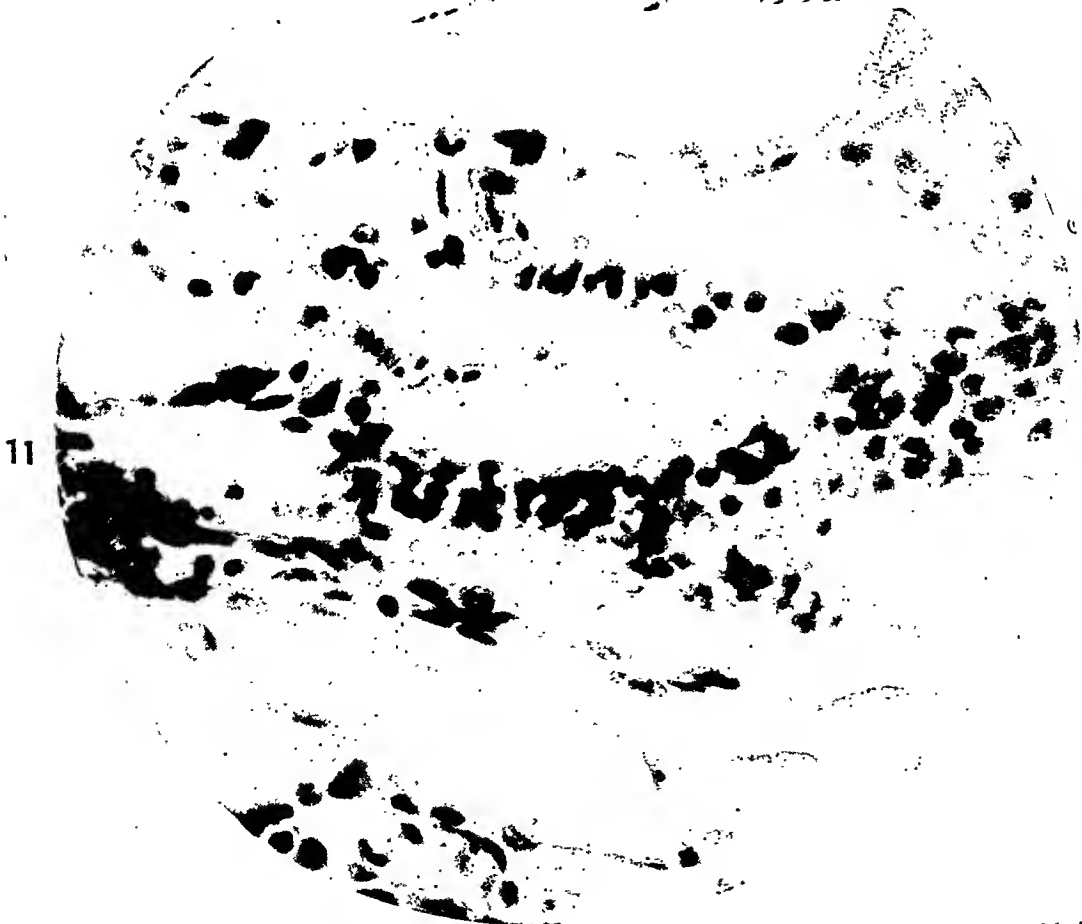


PLATE 24

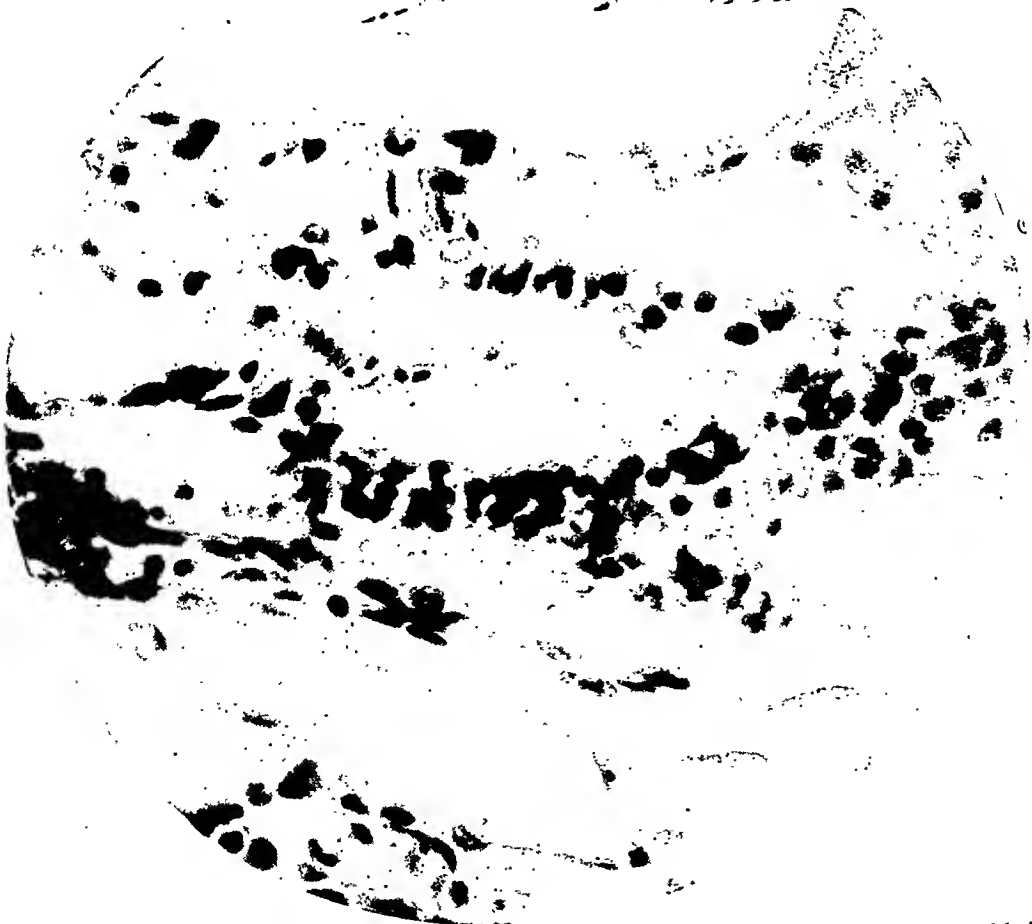
FIG. 10. Case 4. A smaller inflammatory nodule from a specimen from the triceps muscle. There are many scattered mast cells. Cresyl violet stain.  $\times 150$ .

FIG. 11. Case 4. Specimen removed for biopsy from the triceps muscle. Beginning degeneration of a muscle fiber, marked by lighter staining, and central position of muscle nuclei of larger size and having two nucleoli. The perinuclear sarcoplasm is granular and shows beginning vacuolization. The endomysium near this muscle fiber shows infiltration with lymphocytes and plasma cells. Van Gieson's stain.  $\times 675$ .

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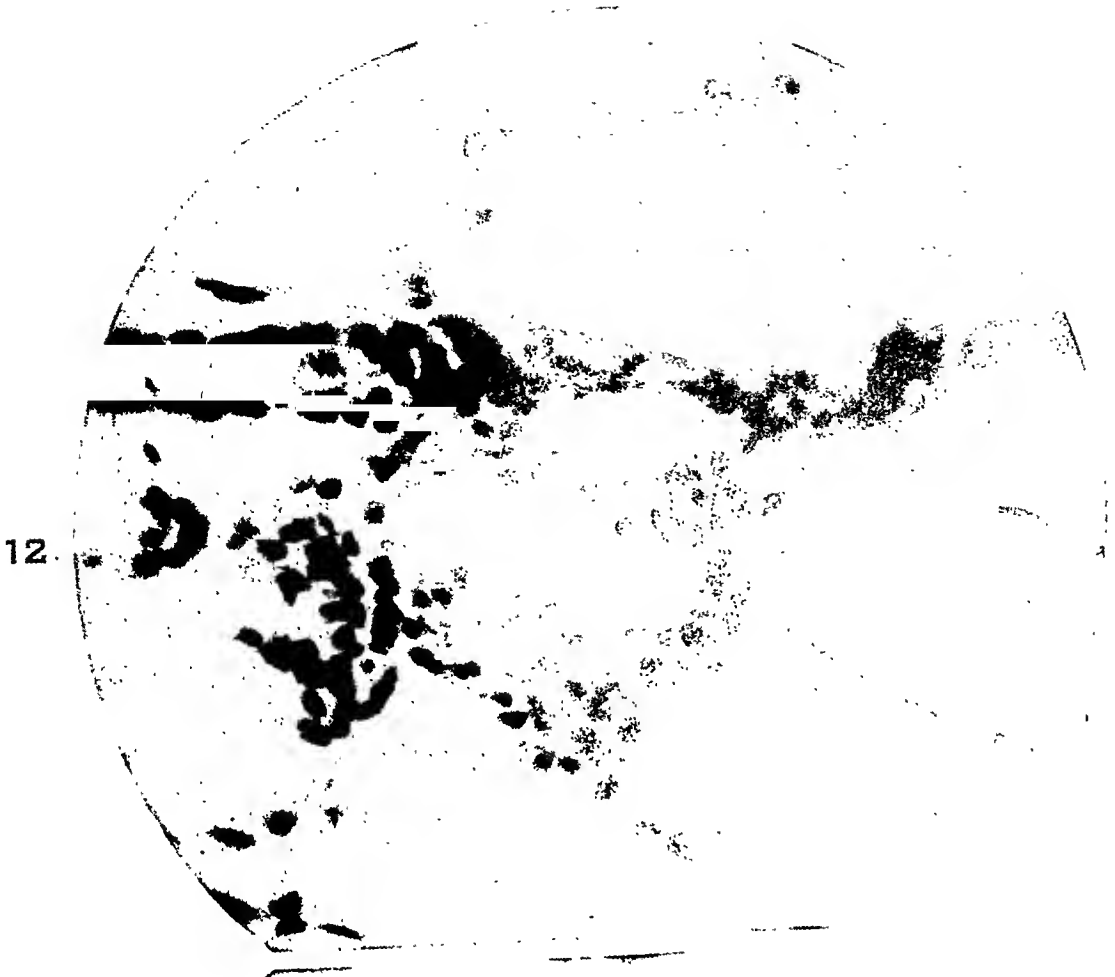
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Skeletal Muscles in Rheumatoid Arthritis

PLATE 25

FIG. 12. Case 4. Specimen removed for biopsy from the triceps muscle. Two muscle fibers, showing degeneration, have a nodular inflammatory reaction around them, and there is a longitudinal arrangement of small vacuoles in the smaller of the two fibers. There are, also, loss of cross striation, and a pale-staining quality. Van Gieson's stain.  $\times 675$ .

FIG. 13. Case 4. Specimen removed for biopsy from the triceps muscle. Abnormal nuclei at the periphery of a degenerating muscle fiber which is surrounded by lymphocytes and plasma cells. Van Gieson's stain.  $\times 675$ .



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Skeletal Muscles in Rheumatoid Arthritis

PLATE 26

FIG. 14. Case 4. Specimen removed for biopsy from the triceps muscle. Two degenerating muscle fibers surrounded by an area of endomysial inflammation. There is a large, dark-staining nucleus in one of the fibers. Van Gieson's stain.  $\times 600$ .

FIG. 15. Case 4. Specimen for biopsy from the triceps muscle. Crossing the field there is a degenerating muscle fiber with coarse cross striations. Below this, a second fiber shows beginning longitudinal fibrillation. This preparation was stained with cresyl violet, which gives injured muscle fibers a deeper blue color than that taken by normal fibers.  $\times 675$ .

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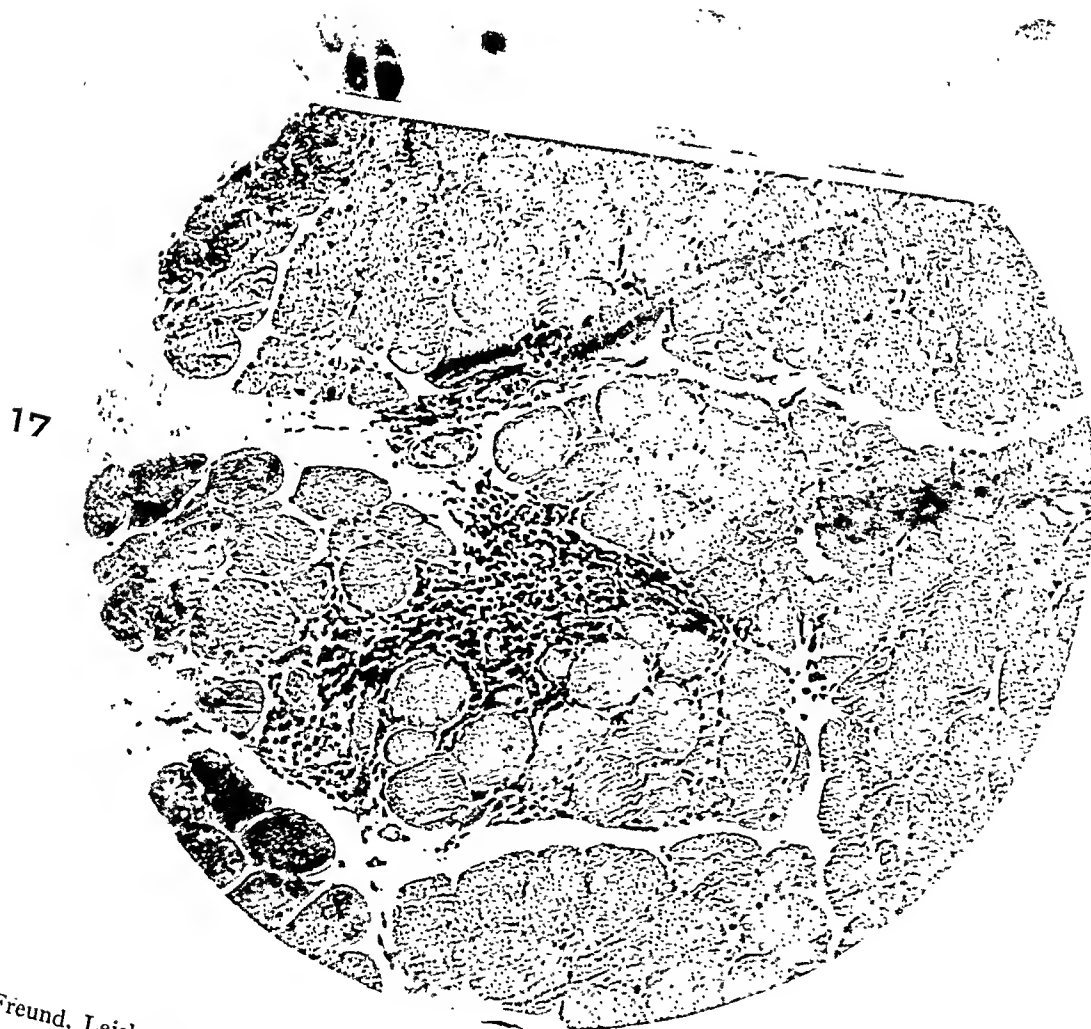
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PLATE 27

FIG. 16. Case 4. Coarse, wavy cross striation of a muscle fiber. Infiltration of lymphocytes and plasma cells. Cresyl violet stain.  $\times 675$ .

FIG. 17. Case 5. Specimen removed for biopsy from the gastrocnemius muscle. Small nodular inflammatory focus in an endomysial location. This shows the extent of infiltration in a transverse plane. Hematoxylin and eosin stain.  $\times 100$ .



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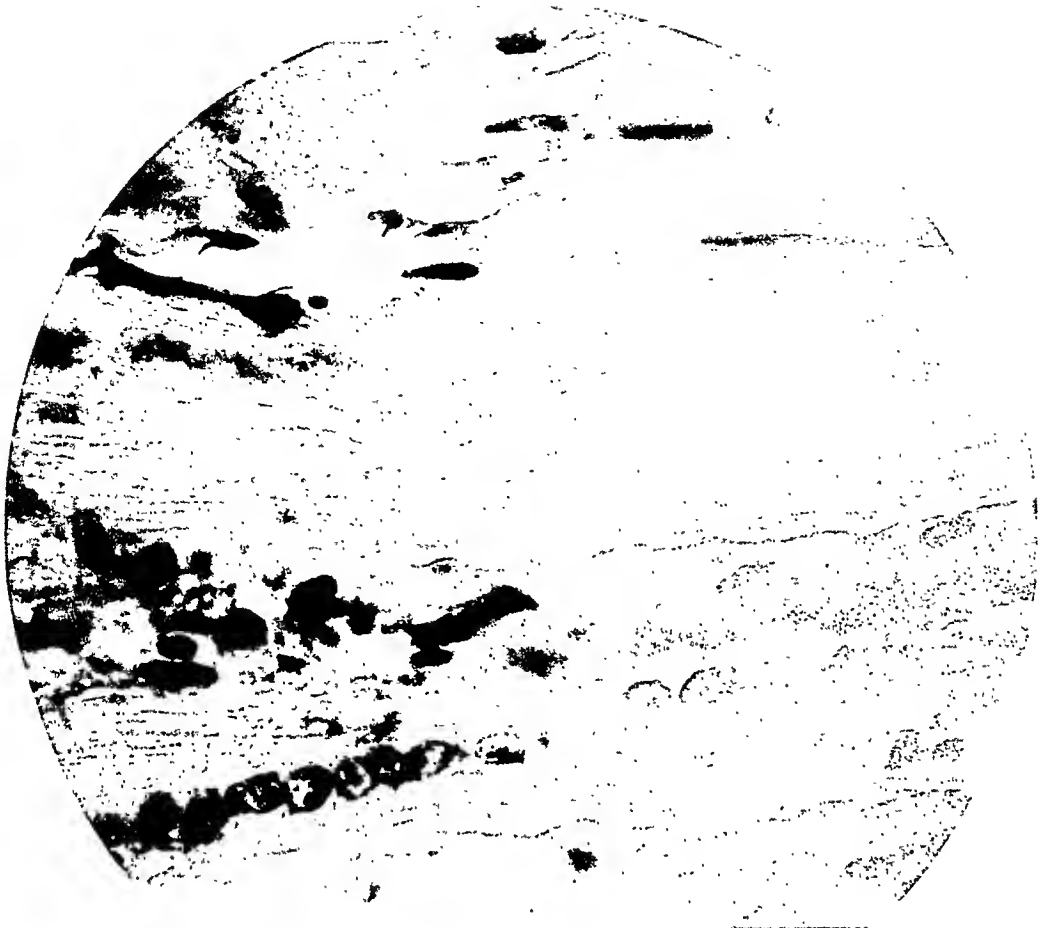
Skeletal Muscles in Rheumatoid Arthritis



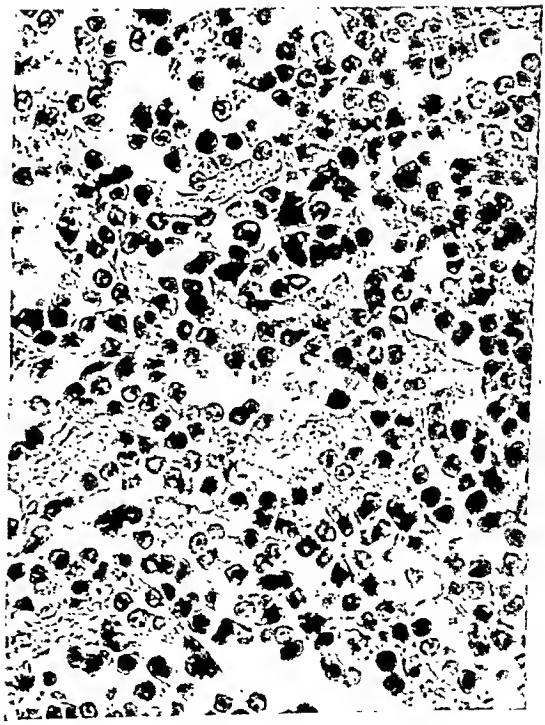
PLATE 28

- FIG. 18. Case 5. Specimen removed for biopsy from the gastrocnemius muscle. A degenerating muscle fiber, with a more centrally placed row of muscular nuclei and vacuolization at the periphery and around the large nuclei, crosses the lower center of the field. Hematoxylin and eosin stain.  $\times 675$ .
- FIG. 19. Case 7. Specimen obtained following amputation of both legs. Muscle tissue near the tibial nerve with a massive inflammatory nodule and severe degeneration and atrophy of muscle fibers. The inflammatory cells are lymphocytes and plasma cells. Hematoxylin and eosin stain.  $\times 100$ .
- FIG. 20. High-power photomicrograph of a portion of the field of Figure 19, showing remnants of degenerating muscle fibers between lymphocytes and plasma cells. Hematoxylin and eosin stain.  $\times 450$ .

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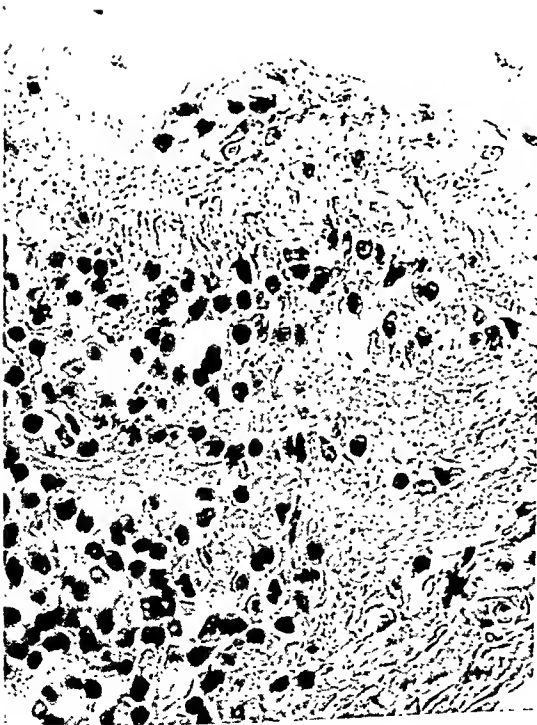
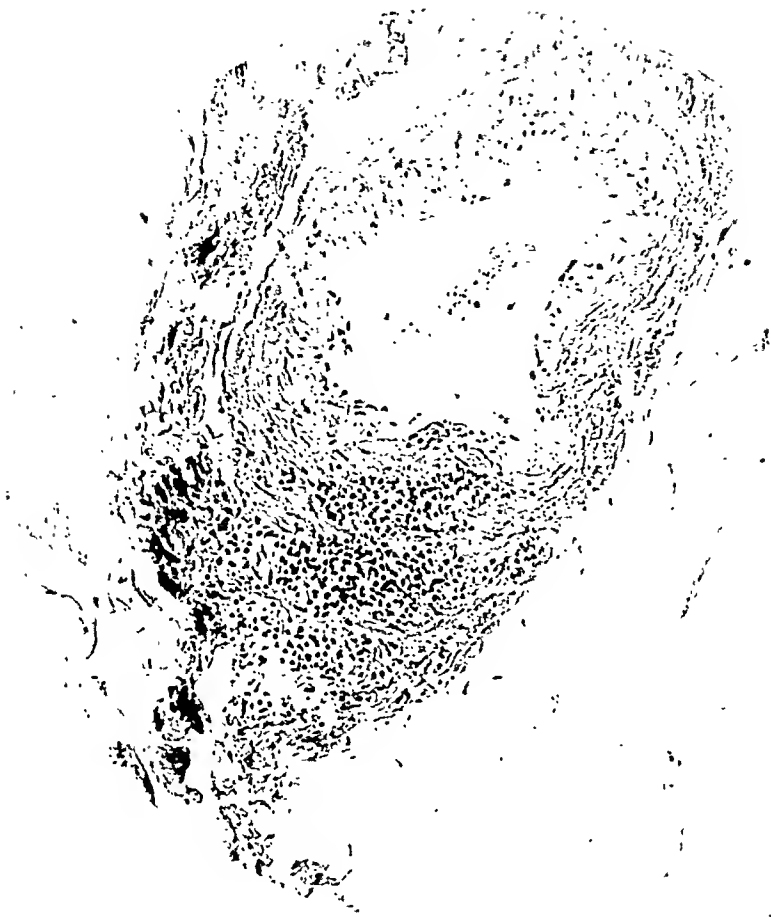
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Skeletal Muscles in Rheumatoid Arthritis

PLATE 29

- FIG. 21. Case 7. Specimen obtained following amputation of both legs. Nodular lymphocytic inflammation in the wall of an artery (media and adventitia), located in perimysium. Hematoxylin and eosin stain.  $\times 150$ .
- FIG. 22. Case 7. A higher power photomicrograph of a portion of the field shown in Figure 21. Hematoxylin and eosin stain.  $\times 450$ .
- FIG. 23. Case 7. Muscle tissue found in the vicinity of the left superficial peroneal nerve. Nearly complete lymphocytic infiltration of the perineurium of a small intramuscular nerve. Hematoxylin and eosin stain.  $\times 150$ .

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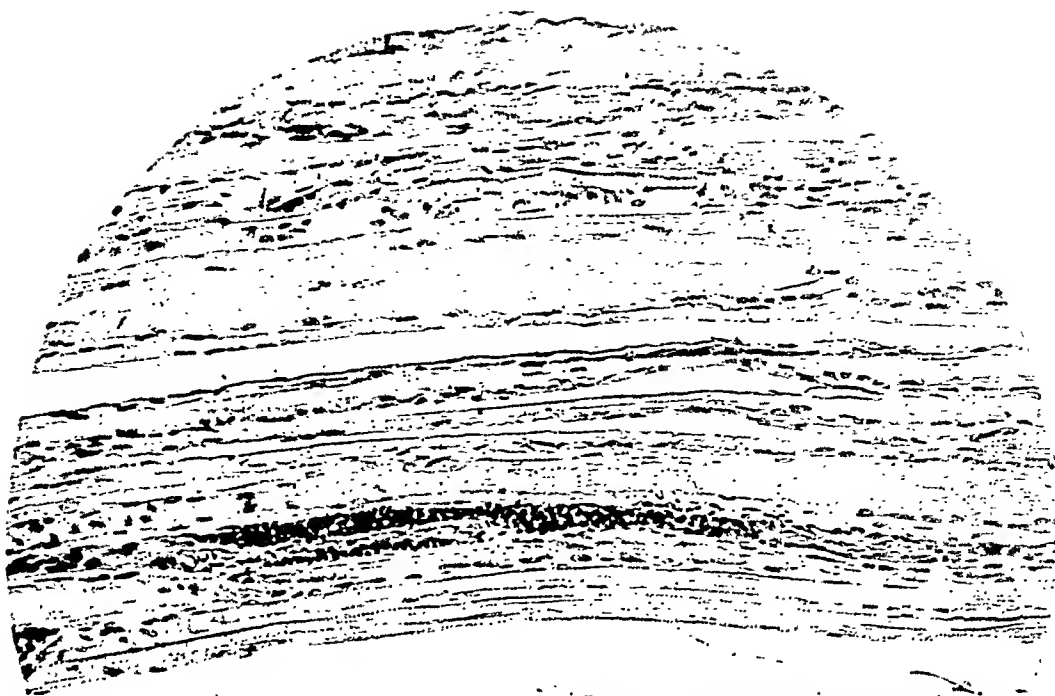
Skeletal Muscles in Rheumatoid Arthritis

PLATE 30

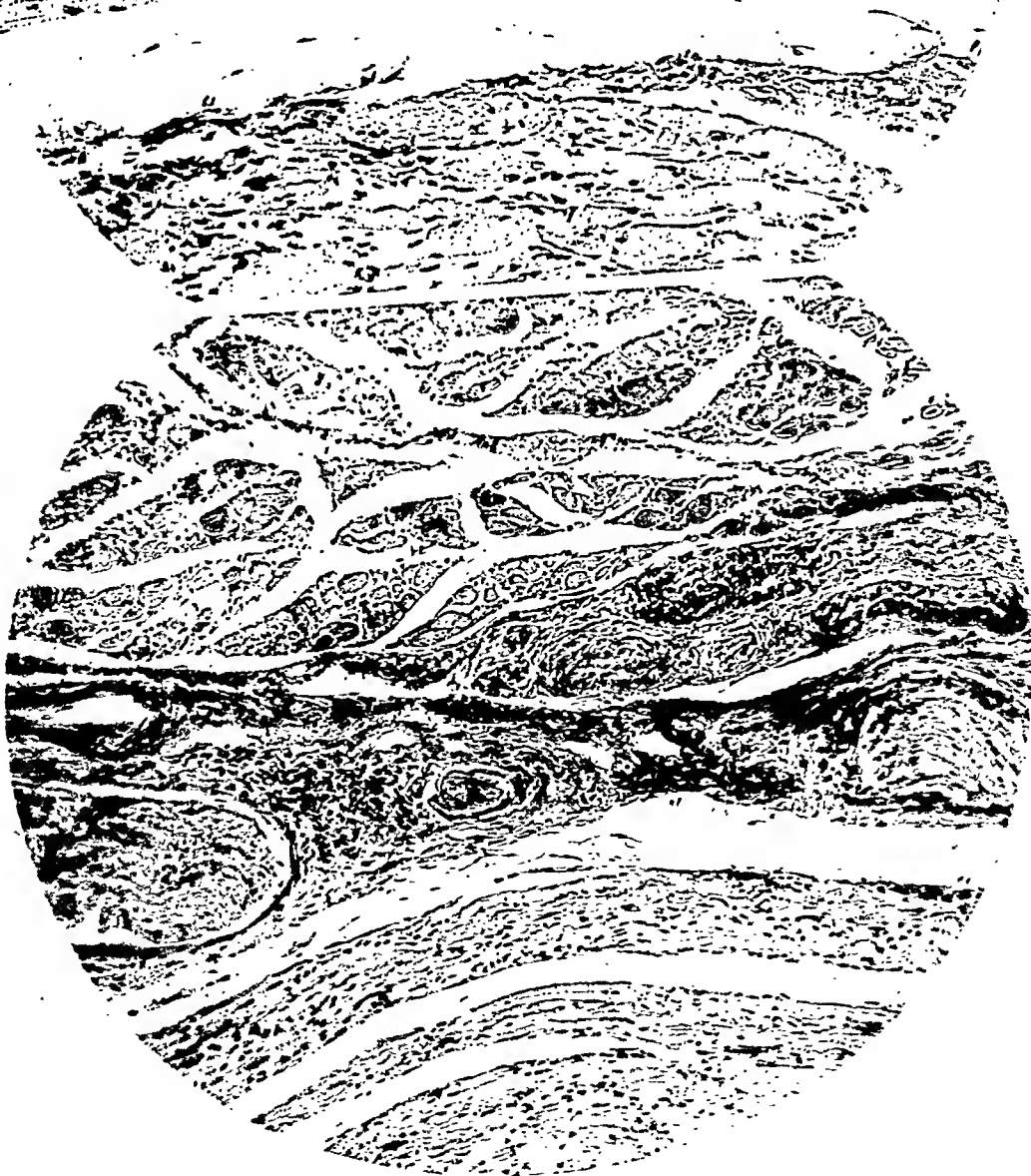
FIG. 24. Case 8. Necropsy specimen from pectoralis major muscle. Small, elongated, spindle-shaped, circumscribed, lymphocytic inflammatory focus in the endomysium. Hematoxylin and eosin stain.  $\times 150$ .

FIG. 25. Case 8. Necropsy specimen from the pectoralis major muscle. Two perimysial inflammatory nodules in periadventitial and perineurial locations. Hematoxylin and eosin stain.  $\times 125$ .

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# HYDROGEN SULFIDE POISONING

## REPORT OF TWO CASES, ONE WITH FATAL OUTCOME, FROM ASSOCIATED MECHANICAL ASPHYXIA \*

A. W. FREIREICH, M.D.†

*(From the Office of the Chief Medical Examiner (Dr. Theodore J. Curphey), Nassau County, N.Y.)*

Poisoning by hydrogen sulfide is a rather unusual occurrence. In certain industries, such as mining gypsum and sulfur, production and refining of high sulfur petroleum, and the manufacture of chemicals, dyes and pigments, hydrogen sulfide is encountered. A group of cases of local and systemic hydrogen sulfide intoxication in workers engaged in excavating in the oolitic limestone of southern Florida has recently been reported.<sup>1</sup>

In large urban centers hydrogen sulfide poisoning is extremely uncommon. Gettler<sup>2</sup> stated that only one such case had been observed by the office of the Chief Medical Examiner of New York City in 25 years.

The following two cases are reported because of the unusual circumstances and in order to acquaint the medical profession and the public with the hazards existing in suburban areas where sewage disposal is by means of cesspools.

### REPORT OF CASES

The cesspool in the rear yard of the house of Mr. A. G., a white male, 42 years old, became filled and was not functioning properly. He engaged a firm of cesspool cleaners to remedy the situation. This was done by pumping approximately half of the fluid content from the pit, which was 8 ft in diameter and 10 ft. deep, and then adding the contents of a large demijohn to the remainder. (The liquid that was added was later found to be commercial concentrated sulfuric acid.) The cesspool was capped and the workers left the premises.

The next morning, Mr. A. G. investigated and found fluid remaining in the cesspool to a level of  $1\frac{1}{2}$  ft. He decided, with the help of his son, A. G., Jr., 17 years old, to empty the remainder with a bucket tied to a rope. When the fluid level became too low for the bucket to be effective, Mr. A. G. lowered himself into the cesspool, filled the bucket, and had his son hoist it up. After a short while in the pit he noticed a smarting of the eyes and returned to the ground level. He entered the pit three times and each time felt a burning sensation about his eyes. After the fourth descent he felt weak and called to his son to "get the ladder down, quick!" He lost consciousness and, on reviving, saw his son lying on the floor of the cesspool. He attempted to move the boy, but again became unconscious. On reviving a second time he called for help and both were removed by neighbors. The father recovered rapidly but the boy remained unconscious and died despite continued artificial respiration.

An autopsy was performed 20 hours after death. On external examination the head and face showed a peculiar greenish blue cyanosis.

\* Received for publication, February 15, 1945.

† Deputy Medical Examiner and Toxicologist, Nassau County, N.Y.; Toxicologist, Meadowbrook Hospital, Hempstead, N.Y.



There were also innumerable small, darker green, circular areas scattered in the greenish blue field (Fig. 1). These spots were located mainly over the left side of the forehead, left cheek and chin. Over the bridge of the nose, across the lips, and on the tip of the chin there were brownish areas having the appearance of corrosive acid burns. The exposed portions of the wrists and hands showed a greenish blue cyanosis similar to that on the face, with darker green, circular areas. The skin of the thighs and legs showed the greenish blue color that is usually seen in advanced decomposition (Fig. 2). The suggillations in some areas were purplish red, but in others were greenish blue.

On making the main incision, pieces of filter paper moistened with freshly prepared lead acetate solution were placed in contact with the pectoral muscles. A faint yellowish brown stain appeared.

When the lungs were removed, a strong odor of hydrogen sulfide was perceived and small bubbles of the gas were seen to escape from the cut ends of the bronchi. Lead acetate paper held near the cut end of the bronchus turned a brown to black color (Fig. 4). On dissecting the bronchi there was seen a large amount of brownish, sandy material mixed with sewage extending to the bronchi of the third and fourth order. Considerable pulmonary edema and congestion were present as well as several large subpleural hemorrhages.

The mouth was filled with brownish, gritty sewage material mixed with sand. The tongue appeared as if burned with acid and a similar picture was seen in the epiglottis, larynx, pharynx, and hypopharynx (Fig. 3). The esophagus throughout its entire length showed a whitish slough on the mucous surface as from acid digestion.

The remainder of the autopsy revealed nothing noteworthy.

Microscopic examination of sections of lung tissue revealed rather marked congestion and exudation of serous fluid into the alveolar spaces. The spleen and kidney showed congestion and the appearance of the other tissues was normal.

Chemical examination of the material removed from the cesspool proved the presence of a large quantity of hydrogen sulfide and free sulfuric acid.

Spectroscopic examination of the blood failed to reveal the characteristic bands of either sulfhemoglobin or methemoglobin.

## DISCUSSION

That sewage which is allowed to putrefy without the access of air is subject to the formation of hydrogen sulfide by the action of bacteria is a well known fact.<sup>3</sup> The sulfides are kept in solution by alkaline salts. The addition of a large quantity of sulfuric acid liberates free hydrogen sulfide.

Hydrogen sulfide is a colorless gas with a specific gravity of 1.192 and an odor as of rotten eggs, familiar to everyone who has worked in a qualitative chemical laboratory. However, a less well known fact is that as the concentration of the gas is increased the odor becomes less apparent, due to paralysis of the olfactory nerves.<sup>4</sup>

In the accident which I have described, there was apparently no awareness of any unusual odors. On being questioned after recovery, the father stated that he did not notice the odor, when it was described to him. Furthermore, when I visited the scene shortly after the boy was pronounced dead, I did not note the odor and it was only after I had walked approximately 50 ft. from the cesspool that the nature of the gas became apparent.

Both the father and son were overcome by the gas. The father became conscious long enough to be saved. The son, however, possibly through the increased respiration engendered by the activity in trying to help his father, became unconscious rapidly and fell face down into the pool of sewage, aspirating the material into his air passages, and finally died as a result of hydrogen sulfide poisoning and mechanical obstructive asphyxia.

The concentrations of the gas in the respired air corresponding to the various manifestations of the poisoning have been determined.<sup>3-5</sup> Table I is that reported by Haggard<sup>4</sup> and is in fairly close agreement with the others.

TABLE I  
*The Toxic Concentrations of Hydrogen Sulfide \**

Concentration of hydrogen sulfide in air			Symptomatology
Mg. per liter	Parts per million	Per cent	
0.14 to 0.21	100 to 150	0.01 to 0.015	Symptoms of local irritation after many hours of exposure.
0.28 to 0.42	200 to 300	0.02 to 0.03	Causes local irritation if inhaled for one hour and slight general symptoms if inhaled longer.
0.70 to 0.97	500 to 700	0.05 to 0.07	Causes local irritation and slight systemic symptoms within one hour. May cause death after exposure of several hours.
1.0	900	0.09	Causes systemic symptoms in less than thirty minutes. May cause death in less than one hour.
1.7	1,500	0.15	Causes death after fifteen to thirty minutes of exposure.
2.0 and over	1,800 and over	0.18 and over	Causes almost immediate death through paralysis of breathing.

\* Reprinted, by permission, from Haggard, H. W. The toxicology of hydrogen sulphide. *J. Indust. Hyg. & Toxicol.*, 1925, 7, 113-121.

The determination of the cause of death by finding hydrogen sulfide in the blood is impossible as the "oxidation is of such rapidity that the gas disappears during the time spent in making the necessary manipulation."<sup>4</sup>

Spectroscopic examination of the blood is unreliable.<sup>6</sup> Sulfhemoglobin has never been found in the blood of persons who have died from hydrogen sulfide poisoning when the examination was made before decomposition had set in.<sup>4</sup> When brought into contact with blood in which the hemoglobin contains oxygen, the hydrogen sulfide is oxidized and no sulfhemoglobin is formed. Only when fresh hydrogen sulfide is brought to act on the blood after complete reduction has occurred, as happens during putrefaction, is a sulfhemoglobin compound formed.

In the fatal case reported here, determination of the actual causative agent was aided by the presence of material containing hydrogen sulfide in the bronchi and examination of the scene shortly after death was reported. The reaction obtained by placing lead acetate paper in contact with the sectioned muscles,<sup>6</sup> while providing valuable corroboration, cannot be used as conclusive proof.

#### SUMMARY AND CONCLUSIONS

Hydrogen sulfide is a very toxic gas. At concentrations at which the odor is apparent, the toxicity is not very great, but when higher concentrations are reached rapid poisoning may occur. The danger becomes greater because of the early paralysis of the olfactory nerves and consequent unawareness of his peril on the part of the subject.

Two cases of hydrogen sulfide poisoning are reported, one with fatal outcome, as a result of attempting to clean out a cesspool to which concentrated sulfuric acid had been added. Health protection agencies and the public should be warned of the dangers inherent in such procedures.

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6. Gonzales, T. A., Vance, M., and Helpem, M. *Legal Medicine and Toxicology*. D. Appleton-Century Co., Inc., New York, 1937, pp. 632-633.

[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

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### PLATE 31

- FIG. 1. In addition to the greenish blue and dark green areas distributed particularly over the left side of the forehead, left cheek and chin, there are brownish areas due to contact with sulfuric acid.
- FIG. 2. Fatal hydrogen sulfide poisoning, with associated obstructive asphyxia. The skin of the face, wrists, hands, thighs, and legs showed a peculiar greenish blue color with small, darker green, scattered, circular areas.

1

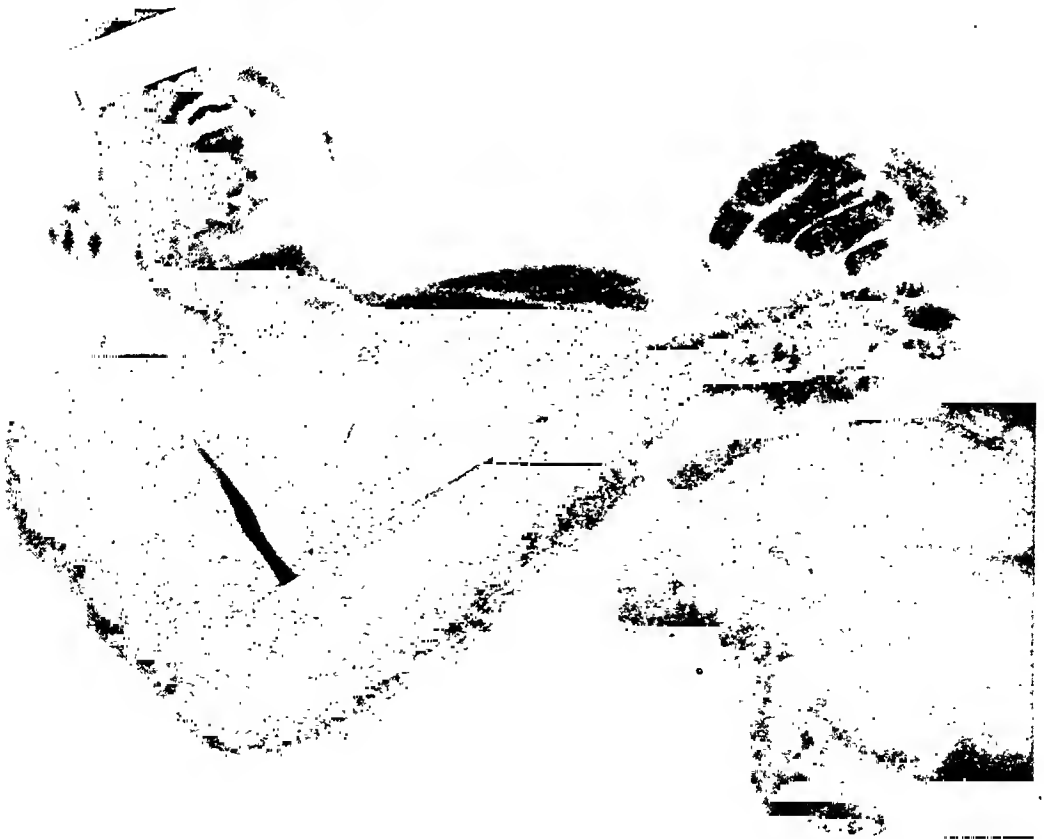
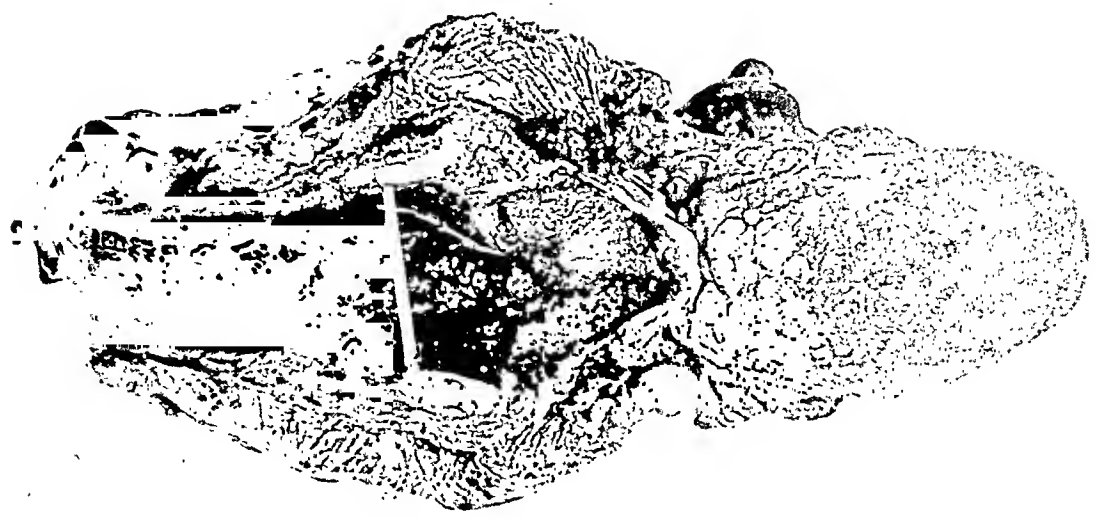


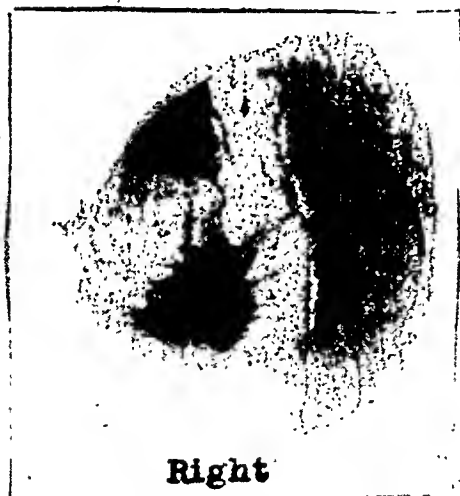
PLATE 32

FIG. 3. Sulfuric acid burns of the tongue, pharynx, epiglottis, and larynx.

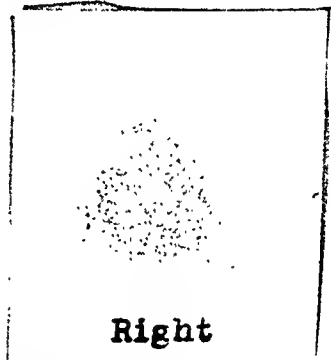
FIG. 4. Stains obtained by holding lead acetate paper near the cut ends of the right and left bronchi.



3



Right



Right



Left

4

Freireich

Hydrogen Sulfide Poisoning





# HYPERPLASIA OF THE ADRENAL CORTEX ASSOCIATED WITH BILATERAL TESTICULAR TUMORS \*

HILLIARD COHEN, MAJOR, M.C., A.U.S.†

Hyperplasia and neoplasia of adrenocortical tissue are commonly associated with profound alterations in somatic and genital development.<sup>1-6</sup> In the prepubertal male these alterations usually are manifested by excessive masculinization, including precocious development of primary and secondary sexual characteristics and increased body growth. As far as can be ascertained, the literature does not contain a single report of proved adrenocortical hyperplasia or neoplasia with macrogenitosomia in the male, in which the patient lived to adult life. Only one instance of a combination of hyperplasia of the adrenal cortex, hyperplastic adrenocortical tissue in the testes, and macrogenitosomia has been previously reported.<sup>1</sup> This patient was a child who died at the age of 3½ years.

I wish to report a case, with autopsy findings, of bilateral hyperplasia of the adrenal cortex associated with bilateral, solitary, testicular masses resembling adrenocortical tissue, occurring in an adult male who in childhood exhibited pronounced precocious genital and somatic development.

## REPORT OF CASE

*Clinical History.* The patient was an acutely ill white male, 20 years old. He was admitted to the Station Hospital, Fort Belvoir, Va., with a 24-hour history of fever, chilliness, headache, right chest pain, nausea, and vomiting. On examination, the temperature was 104° F., respirations were 28, pulse was rapid and barely perceptible, and blood pressure was 60/40 mm. of Hg. Shortly after admission the blood pressure was unobtainable, and the patient died 12 hours later in profound peripheral circulatory failure. The vascular collapse dominated the clinical picture. The clinical diagnosis was lobar pneumonia, lower lobe, right lung, pneumococcus, type 1. Cultures of the blood and spinal fluid were sterile.

A developmental abnormality was suggested by the striking findings in the adrenal glands and testes at post-mortem examination. Through the cooperation of the mother of the patient, valuable records and a snapshot of the patient at the age of 2½ years were obtained from the pediatrician (Dr. Pounders) who had attended the patient from the age of 20 to 35 months. In addition, Mt. Sinai Hospital of Cleveland, Ohio, forwarded a clinical abstract of the examinations of the patient when he was 6 years of age.

When first seen by the pediatrician, the boy was 20 months of age, of normal height—32¾ inches (83 cm.)—and slightly underweight (Table I). He appeared normal in all other respects except for evi-

\* Received for publication, February 19, 1945.

† Now at Army Institute of Pathology, Army Medical Museum, Washington 25, D.C.

dence of slight rickets. No abnormalities in somatic development or in development of the sexual organs were apparent at that time. At a subsequent visit 9 months later (at 29 months of age, Fig. 1), the penis was greatly enlarged, and the pubic hair was about  $\frac{1}{2}$  inch (1.3 cm.) in length. A history of frequent erections was obtained. The height was  $34\frac{1}{2}$  inches (88 cm.), which was still within normal limits for his age. When last seen by the pediatrician the boy was 35 months of age; his height was  $39\frac{3}{4}$  inches (101 cm.), an increase of  $5\frac{1}{4}$  inches (13 cm.) in 6 months. The penis was still distinctly overdeveloped, and frequent erections persisted. The testes were not proportionately enlarged. Radiographic examination of the skull revealed no abnormalities.

TABLE I

*Height and Weight of Patient at Different Ages, as Compared with Approximate Average Normals for the Same Ages*

Age	Height		Weight	
	Patient	Average normal*	Patient	Average normal*
	inches	inches	pounds	pounds
20 mos.	$32\frac{3}{4}$	32		
29 mos.	$34\frac{1}{2}$	$35\frac{1}{2}$		
35 mos.	$39\frac{3}{4}$	37		
6 yrs.	$56\frac{3}{4}$	45	78	44
20 yrs.	61	68	140	135

\* Normal values derived from: Holt, L. E., and McIntosh, R. *Diseases of Infancy and Childhood*. D. Appleton-Century Co., Inc., New York, 1940, ed. 11, p. 22.

At the age of 6, the boy was taken to the Out-Patient Department of the Mt. Sinai Hospital of Cleveland. At that time his height was  $56\frac{3}{4}$  inches (144 cm.), an increase of approximately  $1\frac{1}{2}$  feet (43 cm.) in 3 years, and his weight was 78 pounds (35 kg.). The external genitalia were well developed, and there was a moderate growth of pubic hair. The physical development was that of a boy of 10 to 12 years. The head was "somewhat suggestive of hydrocephalus." Thorough medical, neurologic, and ophthalmologic examinations revealed no further significant findings. There were no pathologic changes in the cranial cavity on radiographic examination. The basal metabolic rate was within normal limits (plus 11 per cent); the glucose tolerance curve was normal; and routine examinations of blood and urine were negative.

Further pertinent information from medical sources was not obtained. According to the mother, the period of rapid growth lasted until the boy was 10 years of age, and very little growth, if any, occurred after the age of 12. At that time he was approximately 5 feet (152 cm.) tall. The mother further stated that the boy's mental de-

velopment kept abreast of his rapid physical development, and "he seemed to have a clearer understanding and a more balanced sense of values than the average boy of his age group." There was no record of a craving for salt or salty foods.

Statements prepared by soldiers closely associated with the patient during his period of army service indicated: (1) that his intelligence was well above average, (2) that he mixed well with the soldiers and was uniformly popular among them, (3) that he apparently did not suffer an inferiority complex because of his short stature, (4) that he overcompensated for his small size by physical vigor, which led on occasions to evidence of extreme tiredness, (5) that he was religious, and closely attached to his mother, (6) that he did not engage in conversations regarding sex to the same extent as most of the other men, and (7) that he had never had sexual intercourse.

#### POST-MORTEM EXAMINATION \*

The body was well developed and well nourished, with a normal but small male physique and with normal male distribution of hair. There was no gross disproportion between length of trunk and of the extremities. The body length was 61 inches (155 cm.). The body weight was estimated to be 140 pounds (64 kg.). The scalp was covered with a scant growth of thin brown hair. No abnormal pigmentation of the skin or mucous membrane was noted. The penis was of average size. Both testes were in the scrotum, and by palpation each seemed smaller than usual. There were no abdominal striae, and excessive subcutaneous fat was not present. The pertinent findings exclusive of the suprarenal glands and testes were:

- (1) Type 1 pneumococcus lobar pneumonia, middle lobe, right lung;
- (2) Persistent thymus of moderate size (35 gm.), moderate lymphoid hyperplasia of the appendix, and slight lymphoid hyperplasia of the spleen. Lymphoid tissue elsewhere was not hyperplastic;
- (3) Cholelithiasis—solitary, soft, brown-black calculus containing a large amount of bile pigments, a trace of cholesterol, and no significant amount of inorganic constituents.

#### *Suprarenal Glands*

*Gross Examination.* Each suprarenal gland (Fig. 2) was approximately five times the average size, the left weighing 50 gm. and the right, 40 gm. Both were firm and of a similar appearance grossly. The cut surface presented a thin, well defined, irregular, bright yellow peripheral zone measuring up to 1 mm. in thickness. Between the ex-

\* Permission was not granted to examine the contents of the cranial cavity.

ternal yellow layers, a thick chocolate-brown zone occupied the central portion of both glands. This zone measured up to 8 mm. in thickness and accounted for the bulk of the glands. In a few of the sections a thin, pale gray medulla was present in the center of the gland; it measured from 0.5 to 1 mm. in thickness.

*Microscopic Examination.* The greater part of each suprarenal gland was composed of a broad, relatively well defined zone of tissue bounded by the medulla internally, and by the remnants of zona fasciculata, and at times zona glomerulosa, externally. The major portion of this hyperplastic zone was made up of cell nests of various shapes and sizes which were separated by intensely dilated and engorged sinusoidal capillaries (Fig. 4). The characteristic cell in most of the hyperplastic zone was rounded or polyhedral and had an average measurement of 16 by 13  $\mu$ . Its cytoplasm was compact, granular, relatively free of vacuoles, and laden with much granular brown pigment. The cells contained one nucleus which was usually eccentric and vesicular; occasionally the nucleus was hyperchromatic. One nucleolus was the rule. The hyperemia and the brown pigment in the cells constituted the two most striking features in this zone.

The peripheral portion of the hyperplastic zone encroached on and, at times, actually replaced the zona fasciculata. It differed from the main body of the hyperplastic zone in the following respects: (1) Though numerous vascular spaces were present, hyperemia was virtually absent; (2) The component cell was larger; the average measurement was 22 by 19  $\mu$ ; and (3) The cytoplasm, which frequently was compact peripherally and rarefied centrally, contained less pigment and was fluffy or finely vacuolated.

In the zona fasciculata many of the normal parallel columns of cells were replaced by the cell nests of the hyperplastic zone. The fascicles which remained, as compared with the normal gland, were considerably diminished in length, and the component cells were distinctly less vacuolated than normal.

The zona glomerulosa was slightly broader than usual; the cells were well preserved and showed no significant histologic changes. Occasionally, cells of the zona glomerulosa were seen to stream far into the subjacent zona fasciculata.

As compared with the normal cortex, there was a striking diminution in the amount of lipoid present in the cells. Although small lipoidal droplets were seen in most of the cells of the hyperplastic zone, they were especially pronounced in the remnants of the zona fasciculata. Oil red O was employed for demonstration of the lipids.

The medulla was without histopathologic alterations.

### *Testes*

*Gross Examination.* The testes were similar in size and appearance (Fig. 3). They were ovoid, firm, and small, approximately two-thirds the usual size. The external surface was smooth and gray-white. Each measured 3 by 1 cm. On cut surface each was composed of a peripheral yellow-gray zone and a central, firm, well defined, dark reddish brown tumor which measured 2 by 1 cm. The tumors were lobulated and contained numerous gray streaks.

*Microscopic Examination.* The testicular tumors showed a similar histologic picture. They were sharply demarcated from the peripherally situated, seminiferous tubular tissue. A definite capsule was not evident. The pattern of the cell groups and the structure of the component cells were uniform. The tumors were composed of cells variously arranged in small groups and as individual elements. The cells and cell groups were separated by a rich network of collagenous tissue strands and bundles, and by innumerable capillaries, many of which were dilated and engorged with erythrocytes (Fig. 5). These engorged vessels frequently assumed the appearance of sinusoidal channels and were especially prominent at the periphery of the tumor lobules. The representative cell of the tumor was rounded or polyhedral and had an average measurement of 20 by 17  $\mu$ . Its cytoplasm, which was pale, granular, and faintly vacuolated, was characteristically condensed at the periphery of the cell and rarefied near the center. Lipids were present in the cytoplasm in only small amounts as fine droplets. In many of the cells a granular, pale brown pigment was also present in the cytoplasm. No crystalloids were seen. The nucleus of the cell was usually prominent, round, vesicular, and somewhat eccentrically located; there was usually a single, distinct nucleolus. Occasionally the nucleus was hyperchromatic. Mitotic figures were not seen. The cells of the testicular tumors bore a striking resemblance to the cells in the outer portion of the hyperplastic adrenal cortex. The vascular pattern of the tumor was quite similar to that which was present in the main body of the hyperplastic adrenal cortex.

In the tumor of the right testis was a small, discrete, almost completely encapsulated, relatively nonhyperemic, adenomatous nodule (Fig. 6). The cytoplasm of the component cells was smooth, homogeneous, and darkly eosinophilic, in contrast to the pale and granular cytoplasm of the cells occupying the tumor proper (Fig. 7). Pigment was not present in the cytoplasm. Generally the cells in this nodule were smaller than those in the main testicular tumor. The average diameters of the cells were 15 by 13  $\mu$ . Large bizarre cells with an eccentric hyperchromatic nucleus were occasionally seen. Where the connective

tissue capsule of the nodule was deficient, the cells of the nodule were continuous with the cells of the main testicular tumor. At times, cells with two nuclei, rarely more, were encountered in the main tumor and in the discrete nodule.

Tubules of the rete testis occurred throughout the tumor. They were most conspicuous at the hilar area and in the interlobular septa of the tumor. Occasional atrophic seminiferous tubules were scattered throughout the tumor.

In the periphery of the testis the seminiferous tubules were strikingly set off from the tumor. They showed a uniform, moderate atrophy. The cells were arranged in one or, at the most, two layers. Evidence of spermatogenesis was wanting. The basement membrane of the tubules was distinctly thickened. The stroma contained an increased amount of loose connective tissue. Careful search of several sections taken from different parts of the testes failed to reveal Leydig cells in the stroma of the peripherally located tubular tissue. The tunica albuginea was of average thickness.

### DISCUSSION

The morphologic identification of the testicular tumors presents the most difficult problem in the study of this case. Although tumors of the testes may originate from any one of several types of cells or tissues, it is possible, without too much difficulty, to limit the origin of the tumors in this case to either interstitial or aberrant adrenocortical cells. It is well recognized that adrenocortical and interstitial cells have many cytologic features in common, and differentiation on morphologic grounds may be difficult.

A review of the interstitial cell tumors reported in the literature<sup>7-16</sup> and a study of several instances of interstitial cell hyperplasia and of one interstitial cell tumor\* reveal certain similarities and certain fundamental differences between interstitial cell growths and the tumors found in my case. These are tabulated as follows:

	<i>Interstitial Cell Tumors</i>	<i>Tumors in Present Case</i>
1. Color	Various colors have been described; usually the tumors are light in color; only occasionally are they dark brown	Dark brown
2. Bilaterality	Only one bilateral interstitial cell tumor has been reported; all others have been unilateral	Bilateral

\* Dr. Paul Klemperer of the Mt. Sinai Hospital, New York City, graciously permitted me to study the slide of an interstitial cell tumor.

3. Pattern of cell growth	In large sheets, masses, or cords; never in small nests or as individual elements	Small nests and individual elements
4. Vascular pattern	Small networks of capillaries between cell groups	Innumerable vascular channels, frequently occurring as dilated and engorged sinusoidal capillaries
5. Brown pigment in cytoplasm of cells	Present	Present
6. Foamy, spongy, or vacuolated cytoplasm	May occur	Present
7. Reinke's* crystalloids in cells	Absent <sup>12,16</sup>	Absent
8. Leydig cells in peripherally compressed parenchymal tissue	Frequently absent	Absent
9. Precocious somatic development and hypergenitalism	May occur	Present
10. Associated hyperplasia of adrenal cortex	Never described	Present

It is apparent, therefore, that the testicular tumors in this case and interstitial cell tumors have many characteristics in common. Nevertheless, it is felt that the evidence favors an origin from adrenocortical rather than interstitial cells. This evidence is based on the fundamental similarity of the testicular tumors and the hyperplastic adrenal cortex in respect to: (1) gross appearance, (2) arrangement of the cell groups, (3) structure of the cells, and (4) vascular pattern. Additional evidence that the tumors of the testes are of adrenal rather than interstitial cell origin may be adduced from the occurrence of a small discrete nodule in the tumor of the right testis; the histologic appearance of this nodule is strongly suggestive of adrenocortical tissue (Fig. 7). It is of interest to note that in not a single reported instance of human interstitial cell tumors has there been described an associated hyperplasia of the adrenal cortex. Though the occurrence of heterotopic adrenocortical tissue in the testis proper is extremely infrequent, such instances have been recorded previously.<sup>1,17</sup> Adrenal rests occur more often outside the parenchymal tissue of the testis or in relation to other portions of the male genital tract.

The histogenesis of the cells in the hyperplastic adrenal cortex, as well as in the testicular tumors, is of considerable importance and interest. The hyperplastic tissue occupies the position of the zona reticu-

\* Reinke, F. Beiträge zur Histologie des Menschen. Ueber Krystalloidbildungen in den interstitiellen Zellen des menschlichen Hodens. *Arch. f. mikr. Anat.*, 1896, 47, 34-44.



laris of the normal adrenal and, further, extends peripherally to encroach on and frequently replace much of the zona fasciculata. Indeed, this tissue has two characteristics in common with the normal reticularis: (1) brown pigment in the cytoplasm of the cells and (2) engorged sinusoidal channels between the cell nests. The first thought, therefore, is that the tissue represents hyperplastic zona reticularis.

In recent years the concept of a specific androgenic tissue in the adrenals, functionally and anatomically distinct from the permanent cortex or interrenal tissue, has been advanced by Grollman.<sup>18</sup> That these two tissues are distinct is further suggested by the embryologic studies of Keene and Hewer<sup>19</sup> and Uotila.<sup>20</sup> Under normal conditions in man, this so-called androgenic tissue, or, as it is usually termed, the fetal cortex, is abundantly present in the adrenal during fetal life. However, during the first year of extra-uterine life it almost completely disappears. At the height of its development, it is a conspicuous, intensely hyperemic zone which lies between the medulla internally and the tissue destined to become the permanent cortex externally. Grollman believed that the adrenocortical tumors and hyperplasias responsible for virilizing changes in the female, and precocious somatic and sexual development in the prepubertal male, represent a persistence and subsequent overgrowth of the androgenic tissue, rather than a proliferation of the interrenal tissue. In my case the location of the hyperplastic tissue, its pronounced vascularity, and the macrogenitosomia during the patient's childhood strongly suggest that this tissue is androgenic rather than hyperplastic zona reticularis. Unfortunately, there are at present no exact morphologic criteria for differentiating androgenic cells and cells of the zona reticularis.<sup>21</sup> Broster and co-workers<sup>2</sup> believed that the ponceau fuchsin stain is specific for androgenic cells. This stain was used, but negative results were obtained on both the adrenals and testes. Hormone assays were not carried out on the tissues, blood, or urine.

The final problem which confronts us in this case is the rapidly developing and profound peripheral circulatory failure. In the recent literature there are two reports which indicate that functional adrenocortical insufficiency may occur in association with hyperplasia of the adrenal cortex. In a case of macrogenitosomia precox reported by Wilkins *et al.*<sup>1</sup> there were clear-cut clinical and blood-chemical findings of adrenocortical insufficiency in the form of: (1) pigmentation of the skin and mucous membrane, (2) a craving for salt, (3) sudden death, (4) high blood nonprotein nitrogen, and (5) low blood sodium. Anatomically the adrenals showed widespread loss of normal interrenal substance and replacement of the same by hyperplastic tissue, which is

considered to be androgenic tissue. Their interpretation of these changes is that due to a loss of functioning interrenal tissue, adequate hormone was not present to sustain life. Dijkhuizen and Behr<sup>22</sup> reported a series of four cases of pronounced bilateral adrenal hypertrophy (cortical) in young infants, in all of whom the most prominent symptom was severe protracted vomiting followed by death. In three cases the feces were described as thin and green. In all four instances the symptoms were suggestive of intestinal stenosis. An autopsy was performed on three of the patients, and a laparotomy on the fourth. Stenosis or any other abnormality of the gastrointestinal tract could not be demonstrated. In the three cases upon which necropsies were performed, bilateral hypertrophy of the adrenals due to cortical hyperplasia was found. Although post-mortem examination was refused in the fourth case, the authors had reason to believe that adrenal hyperplasia was present likewise. Severe vomiting and other gastrointestinal disturbances are common symptoms of adrenal insufficiency in man and certain animals.<sup>18</sup> That lesions replacing a significant portion of the interrenal tissue, notably the zona fasciculata, may cause adrenal insufficiency is further suggested by Bennett's histochemical studies,<sup>23</sup> which indicate that in the cat, the cortical hormone is localized in the zona fasciculata. Since, in my case, there is adequate evidence of severe encroachment on, and actual replacement of, appreciable portions of the zona fasciculata, I think that functional adrenal insufficiency offers the most reasonable explanation for the circulatory collapse. Circulatory failure is commonly encountered in cortico-adrenal insufficiency.

It is interesting to reconstruct the possible sequence of changes which occurred in the life of this patient as the result of the adrenal and testicular lesions. His infancy was apparently normal. In early childhood the androgenic effect of the lesions became clearly manifest in the form of precocious development of the primary and secondary sexual characteristics and increased body growth. The history suggests that the excessive androgenic stimulation continued until the boy was at least 6 years of age. Beyond this age our knowledge of further somatic and sexual development is incomplete. It is noteworthy that at the age of 6 the patient's body length was  $56\frac{3}{4}$  inches (144 cm.), whereas at the time of death (at the age of 20) he was only 61 inches (155 cm.) in height. It is evident, then, that minimal body growth occurred after the age of 6. Precisely what mechanism effected the relative cessation of continued somatic and sexual development after the age of 6 is not apparent. It seems probable that the epiphyses closed early in life, and this precluded the possibility of further in-

crease in height. Suggestive evidence that a relative functional cortico-adrenal insufficiency existed prior to the terminal infection came from soldiers who knew the patient well. They stated that he tired from physical activity more easily than his associates; the tiredness at times was extreme. Recent studies<sup>24</sup> indicate that hormones of the adrenal cortex play a significant rôle when the organism encounters or is subjected to situations of stress. The adrenocortical insufficient state in man as well as in experimental animals is characterized by weakness, exhaustion, and ultimately by collapse. Further, fatigue is accompanied by an increased urinary output of the 17-ketosteroids, the primary source of which is thought to be the adrenal cortex.<sup>24</sup> It seems reasonable to assume, therefore, that the patient had enough functioning interrenal tissue to meet ordinary needs. When the demands became excessive (physical exertion, severe infection), inadequate amounts of cortical hormone were available, and a state of insufficiency developed.

#### SUMMARY

A case is reported of pronounced bilateral hyperplasia of the adrenal cortex associated with bilateral testicular tumors in an adult male. In childhood the patient exhibited precocious genital and somatic development, whereas in adult life he showed no apparent abnormalities except for small stature. He died in the course of pneumonia (pneumococcus, type 1), middle lobe, right lung. One of the outstanding features of the clinical course of the infection was a rapidly developing and severe circulatory collapse. Histologic study suggests that the testicular tumors were derived from aberrant adrenocortical tissue rather than from interstitial cells. It is thought that peripheral circulatory failure was probably due to cortico-adrenal insufficiency.

I wish to express my gratitude to Dr. Carrol M. Pounders of Oklahoma City, Oklahoma, and to Mt. Sinai Hospital, Cleveland, Ohio, for providing clinical notes; and to the resident civilian consultants and to the regular staff of the Army Medical Museum for their advice and assistance in the study of this case.

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[ Illustrations follow ]

## DESCRIPTION OF PLATES

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### PLATE 33

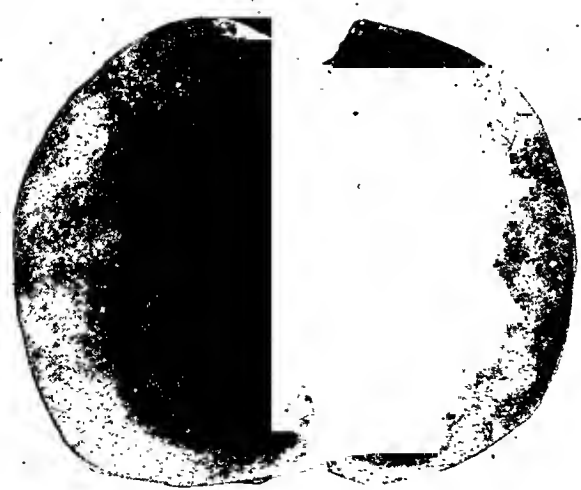
- FIG. 1. A case of hyperplasia of the adrenal cortex associated with bilateral testicular tumors. Photograph of patient at 29 months of age. The penis is enlarged. The pubic hair cannot be seen.
- FIG. 2. Gross appearance of suprarenal glands. In the upper part of the photograph is the cross section of a normal suprarenal gland for comparison. Below it are two representative cross sections of the suprarenal glands in this case. Of note are the pronounced enlargement of the glands, the dark inner hyperplastic zone, and the encroachment of the latter on the peripheral yellow zone (fasciculata). The medulla is not present in either of these sections.  $\times 1\frac{1}{2}$ . A.M.M. negative no. 74988.
- FIG. 3. Gross appearance of left testis. The testis has been bisected through its long axis, exposing both cut surfaces. The central dark tumor is sharply demarcated from the pale, peripheral, testicular tissue.  $\times 2$ . A.M.M. negative no. 74989.



1



2



3

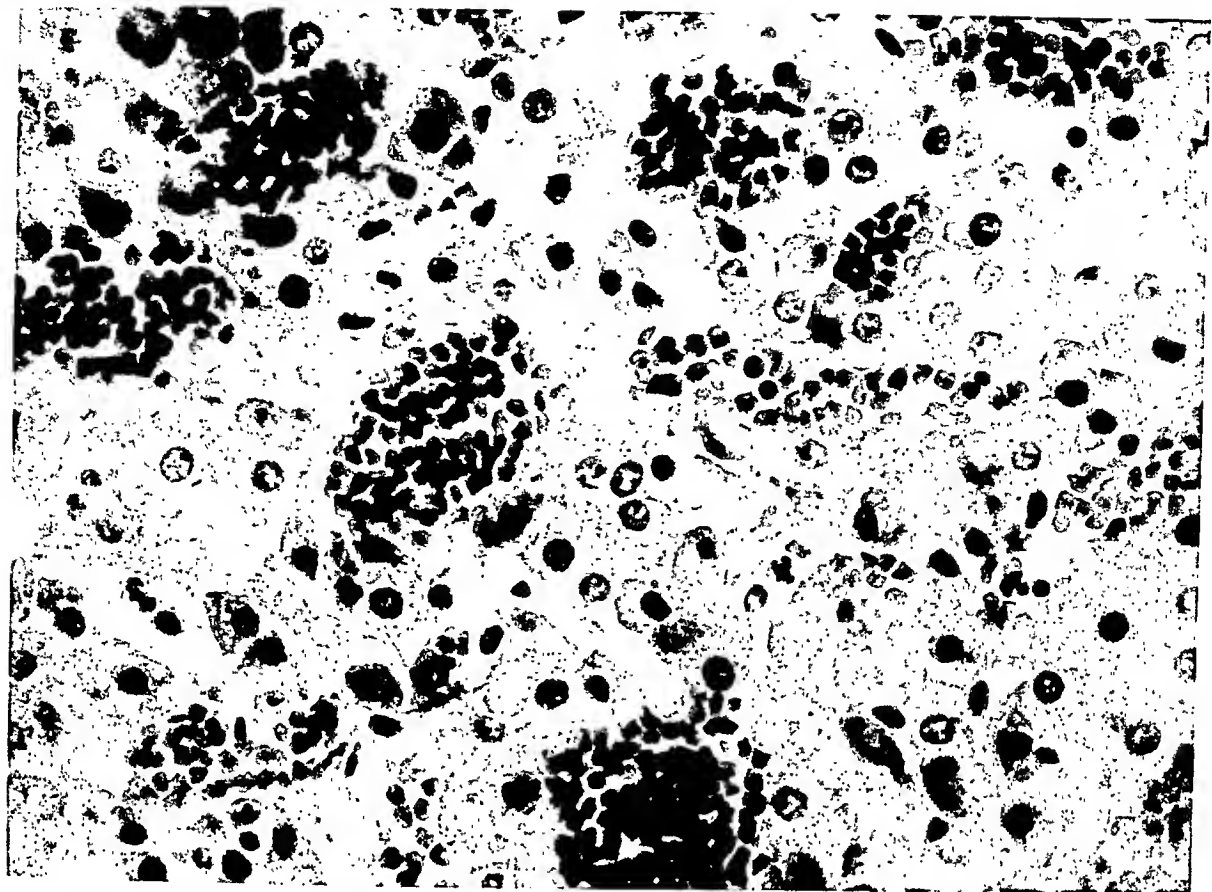
Adrenal Hyperplasia with Testicular Tumors

PLATE 34

FIG. 4. Suprarenal gland, showing inner portion of hyperplastic zone. Of note are the dilated and engorged sinusoidal capillaries and the irregular groups of cells between them.  $\times 515$ . A.M.M. negative no. 77624.

FIG. 5. Testicular tumor, showing the similarity of the vascular pattern and of the arrangement of the cell nests to the hyperplastic adrenal cortex. In the testicular tumor the cells are slightly larger and the cytoplasm more granular than in the inner portion of the hyperplastic zone of the adrenal cortex shown in Figure 4. The peripheral condensation and central rarefaction of the cytoplasm are evident. The blood vessels have prominent endothelial cells.  $\times 515$ . A.M.M. negative 77553.

4



5

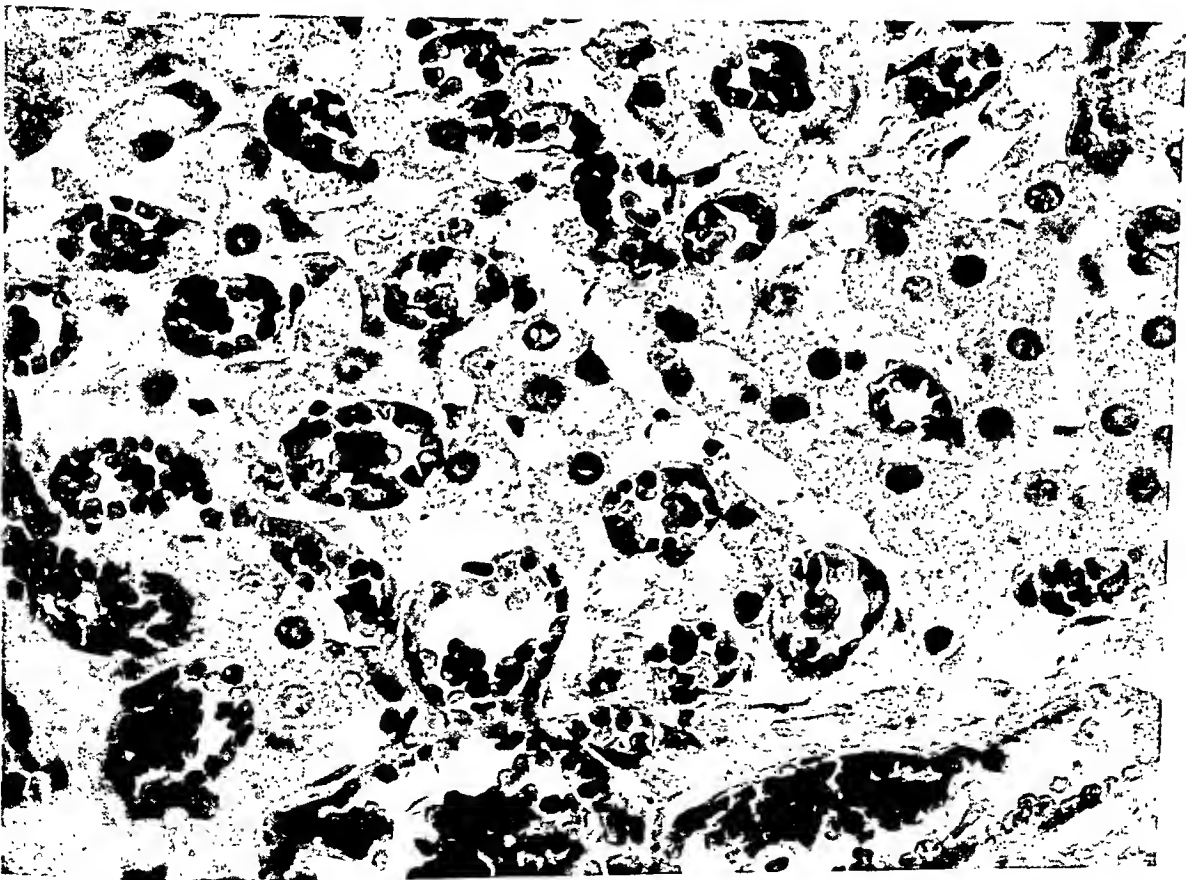


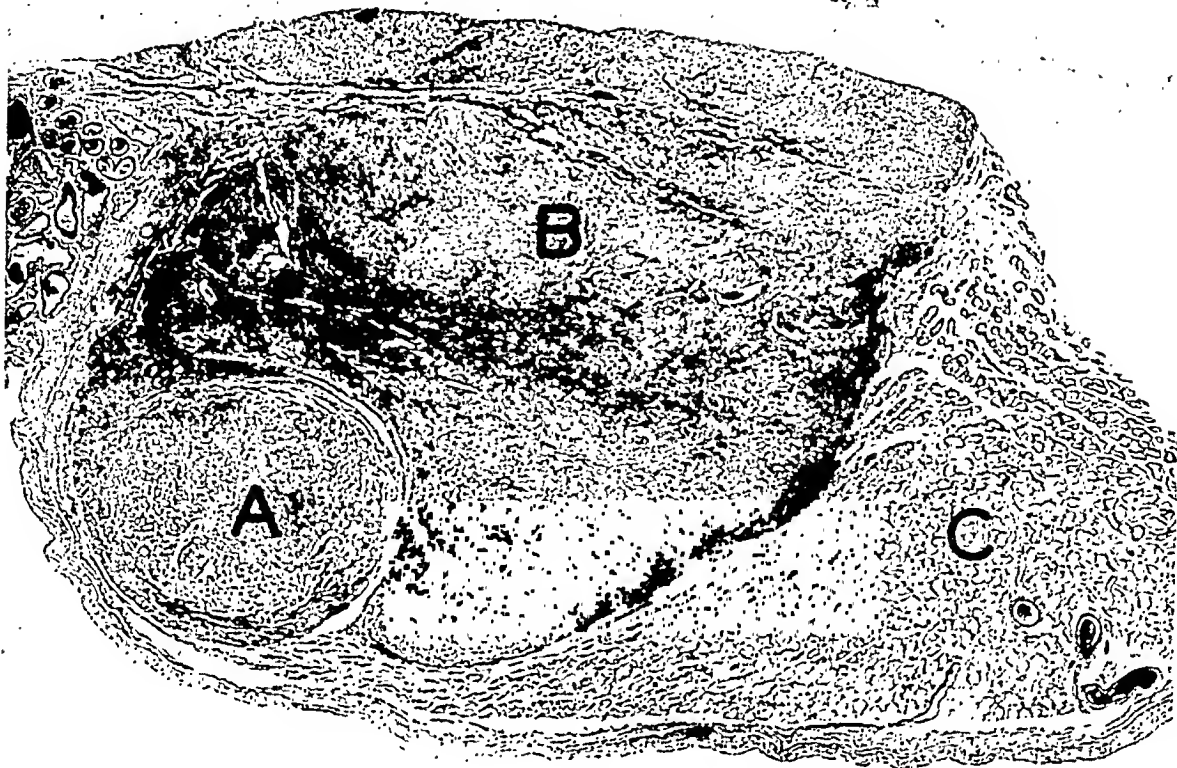


PLATE 35

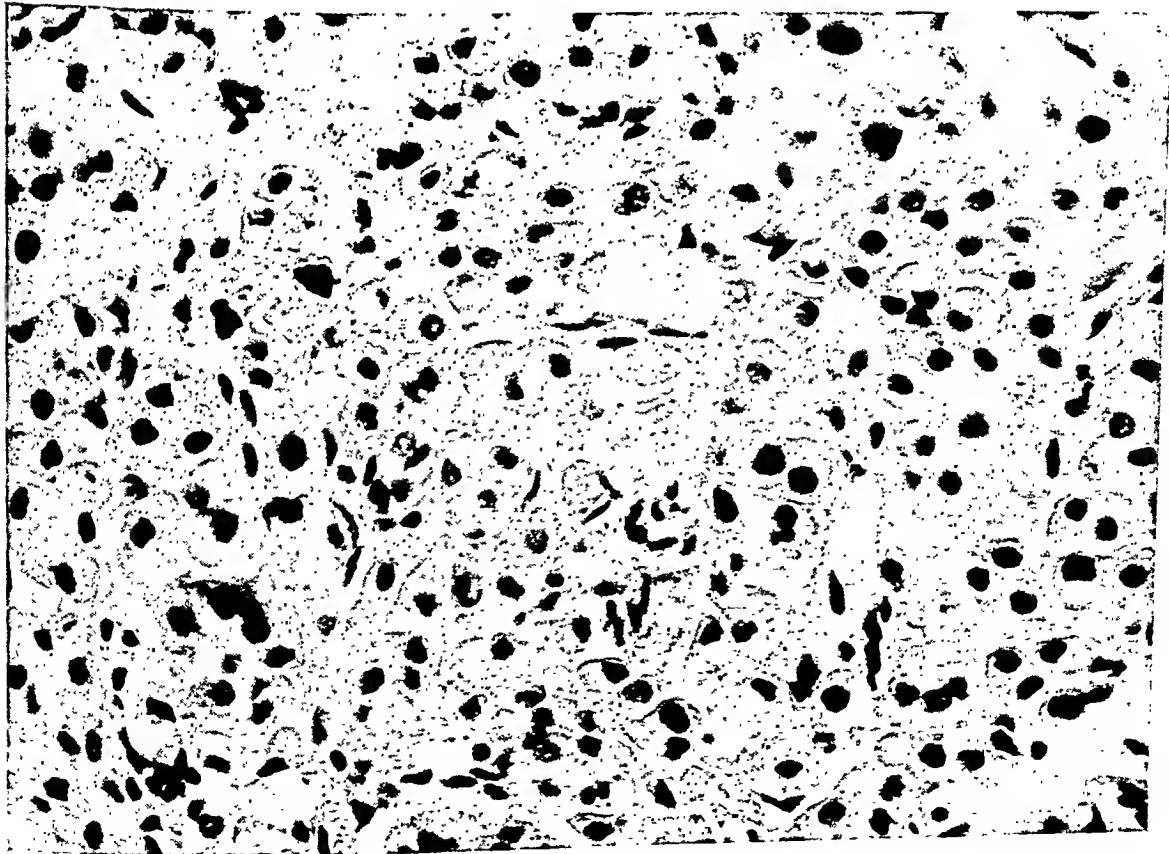
FIG. 6. Small, sharply circumscribed adenomatous nodule of adrenocortical tissue located within main tumor of right testis. (A) Discrete nodule. (B) Main tumor. (C) Atrophic parenchyma of testis.  $\times 10$ . A.M.M. negative no. 77621.

FIG. 7. High-power view of discrete nodule shown in Figure 6. The relative avascularity of the nodule and the similarity of the component cells to adrenocortical cells are apparent.  $\times 515$ . A.M.M. negative 75793.

6



7





# STUDIES ON AMEBOID MOTION AND SECRETION OF MOTOR END-PLATES

## VII. EXPERIMENTAL PATHOLOGY OF THE SECRETORY MECHANISM OF MOTOR END-PLATES IN THERMAL SHOCK \*

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Milwaukee 3, Wis.)*

In reference to the shock of heat stroke, Moon<sup>1</sup> made the following statements (page 366): "The mechanism by which heat stroke causes circulatory death is not known, but many believe it to be through effects upon the central nervous system. Neither is it known whether disturbances in the central nervous system, either organic or functional, affect the circulation through the sympathetic system or through some other route. But it is evident that the same cycle [in the production of shock], whose effects have been seen in numerous other conditions, is operative here."

After years of study of the problem of shock, Cannon<sup>2</sup> clearly stated the unsolved problem of the onset of shock as follows (pages 140 and xi, respectively): "The mode of action of the initiating agent in secondary shock is thus left unsatisfactorily explained." "The reader should understand from the beginning that the mystery of the onset of shock has not been definitely cleared away despite a considerable increase in our knowledge of it, and that there still remains much work to be done before we shall have elucidated all the factors which play a rôle in its establishment." This is particularly true of the rôle of the pathologic motor end-plates in both primary and secondary shock. Cannon and his colleagues did not use morphologic methods in the study of shock. Moon and his associates did employ anatomic methods but did not study the changes in the motor end-plates.

The present study is an attempt to reveal, by the methods of experimental morphology, gold impregnation, and teasing of the innervation of muscle tissue, (1) the nature of the normal mechanism of secretion in motor end-plates of skeletal muscle; and (2) the rôle of this exaggerated secretion in the structural exhaustion of the motor end-plates and alteration of muscle structure and function in the pathologic mechanism of shock produced by heat.

\* This investigation was carried out with the aid of a grant to the Department of Anatomy, Marquette University School of Medicine, by the Baruch Committee on Physical Medicine.

Received for publication, December 18, 1944.

## MATERIALS AND METHODS

The preconception of the histologists, that there is a well defined and constant line of demarcation between nerve and muscle, determined the selection of histologic technics employed in the past in the study of the neuromuscular apparatus. The gold impregnation and teasing method had been replaced by some histologists for those in which silver and methylene blue were used. The granules of Kühne were poorly portrayed by silver and methylene blue but were clearly defined with gold. When there was variation in the intensity of impregnation of the axons and granules with the uncontrolled gold solutions, the method was discarded as unreliable. The fact that impregnation with gold and teasing whole mounts of muscle fibers revealed both the variations in the total morphologic structure and variations of intensity of impregnation of motor end-plates stamped this method as superior to any other. When normal and abnormal tissues were subjected simultaneously to the same solutions, the variations were clearly visualized and could be evaluated.

These changes in form and quantity of nervous substances were, therefore, intrinsic to the tissues and were not due to extrinsic variables in the gold technic. The apparent unreliability and real variations of results misled histologists, and resulted in their discarding a technic that contained the clue to the function of the motor end-plates. Technicians were hunting for a standardized result. The motor end-plates could not be standardized with a constant and fixed structural pattern any more than the cross striations of muscle could be arbitrarily classified with a fixed number of constant sarcomeres. The tissues may be overstained or understained with gold, but under controlled conditions this is avoided, or may be detected and evaluated.

The minute differences of structure and the variable affinity of the neuromuscular apparatus for gold used under the conditions in which comparisons were made, revealed these important facts about the motor end-plate: (1) The pleomorphism is due to ameboid motion; (2) The quantitative variation of granules is due to a secretory mechanism by which the terminal axons of the end-plate are attenuated or depleted by their direct transformation into granules discharged into the myoplasm of the muscle fiber. It was an error, therefore, to consider the junction of nerve and muscle as possessing a fixed structure when, in reality, the relationship was one of elusive continuity rather than that of a deceptive discontinuity. It was no less an error to discard the best method available because of variations of morphologic results, which portrayed the secretory mechanism of the motor end-plates.

The revelation of the gland-like nature of the structure of the motor end-plates is dependent upon the methods selected to demonstrate the polarity of the secretory mechanism of the neuromuscular apparatus. There are several methods commonly used for the microscopic study of the motor end-plates, *viz.*, the silver, the methylene blue, and the gold method. The authors of current histologic textbooks and manuals of technic claim that the gold method is uncertain and unreliable. There is considerable variation of opinion as to which method is most accurate. Experience counts for much in accuracy. The gold method may be easily mastered: our freshmen medical students use it with good effect as a routine laboratory procedure. Impregnation with silver and *intra vitam* staining with methylene blue do not visualize the granules of Kühne as well as does impregnation with gold chloride. Attempts to study the end-plates in living muscle are now in process in our laboratories. This is a difficult problem because of the similarity of the refractive index of living nerve endings and muscle. There appears to be a more specific affinity of the axoplasm and its discharged granules for gold than for any other substance used thus far in the staining or impregnation of the end-plates. *In vitro*, gold chloride forms a specific precipitate with both choline and acetylcholine.

Teasing is better than sectioning for the preservation of the polarity of the secretory mechanism of the motor end-plates. By the teasing method significant changes in the epilemmal axon, hypolemmal ramifications, granules of Kühne, and in the cross striations of muscle may be observed as a whole, simultaneously and in relationship one to another. This structural relationship may be photographed. This total anatomic relationship of nerve and muscle is obscured, if not destroyed, by sectioning the muscle. Gold impregnation and muscle teasing are old methods and therefore are discredited in the minds of a few investigators who possess inadequate experience with them. For the purposes of this study, nevertheless, these methods are superior to any other neurologic technic. The nuclei may be counterstained. The Bielschowsky<sup>3</sup> and Boeke<sup>4</sup> silver and sectioning methods are good for clear visualization of the nuclei and of the variable neurofibrils formed by the streamlining of protoplasmic flow in the axis cylinder. These methods were used as a check against the gold chloride method of impregnation and teasing of muscle. The Spielmeyer technic was used to detect demyelination of the peripheral innervation of muscle and staining with scharlach R for acute fatty degeneration of nerve fibers.

White rats (*Mus norvegicus*) were subjected to shock due to scalding by the method of Prinzmetal, Hechter, Margoles, and Feigen.<sup>5</sup> This method possessed the following features: the degree of trauma

was objectively controlled and reproducible; the method was simple and fast, so that a sufficient number of animals yielded results of statistical significance; finally, the shock-producing procedure resembled a type of trauma which produces shock in man.

The method consisted in immersing the entire body surface, except the head and neck, of either etherized or nonetherized rats, for definite periods of time in a water bath set at different water temperatures. The degree of trauma was a function of the duration of exposure and of the temperature of the bath.

Two hundred rats, average weight 200 gm., were subjected to immersion for 10 seconds at 75° C. The animals were not allowed access to food or water after immersion. At 30-minute intervals the gastrocnemius muscles were excised from 40 of the most prostrated animals, prepared by the gold chloride technic,<sup>6</sup> and teased. Preparations were stained for the study of the myelin sheaths of the epilemmal axons.

Fifty rats were subjected to a temperature of 90° C. The mean survival time was 75 seconds. After immersion 10 of the most prostrated animals were selected at 15-second intervals and their gastrocnemius muscles were excised. The muscles were immediately subjected to the gold technic.

At different time-intervals after the rats were immersed at 75° C. for 10 seconds the temperature of the skin decreased and hemoconcentration was evident, as well as dyspnea. Great muscular weakness and flaccid paralysis were present in later stages. Rats subjected to 90° C. for 10 seconds became spastic at once and exhibited extensor rigidity preceded by muscular twitchings. This pathologic functional response of immediate spasticity of the muscles was exhibited, likewise, by 20 rats immersed in water at 90° C. for 1 second. The temperature of the muscle of these animals varied from 37.8° to 42.4° C.

Twenty rats, average weight 200 gm., were used as controls. The gastrocnemius muscles were excised 15 minutes after the intraperitoneal injection of nembutal, 4 mg. per kg. These relatively normal control muscles were run through the same solutions concurrently for the same periods of time as those muscles excised from the scalded animals. The histologic variations of the motor end-plates are due, therefore, to factors intrinsic to the muscle, and not to uncontrolled accidental variations of technic extrinsic to the muscle. The simple and easily reproducible experiments under controlled conditions herein outlined may be used as classroom demonstrations of the secretory mechanism of the motor end-plates in skeletal muscle.

Since photographic evidence is the only acceptable means of over-

coming the current confusion about the true structure of the motor end-plates in shock, an apparent abundance of illustrations is presented. On the other hand, all that has been written on this subject for over a century has convinced no one. This photographic series constitutes objective evidence which any one may easily observe and as easily evaluate. Thus a permanent atlas is at hand to serve anyone as a point of departure for future experiments on the pleomorphism of the neuromuscular apparatus.

Small variations from the normal, standard intensity of the impregnation with gold may be attributed by some critics to faulty technic of overstaining or understaining and not to any irregularity in the quantity and structure of the chrysophilic axonic substances in the end-plate itself. Since normal and abnormal tissues were subjected concurrently to the same concentrations of solutions and for the same periods of time, these differences are due to variations in the quantity of the axonic substances in the motor end-plates themselves. These minute differences in the quantity of Kühne's granules, and variations in the form and structure of the hypolemmal axons of the motor end-plates, offer real footholds for scientific advance in the anatomy, physiology, and pathology of the neuromuscular apparatus.

## EXPERIMENTAL OBSERVATIONS

### *I. The Pathologic Physiology of Muscle in Thermal Shock*

There were variations not only in the time of onset but also in the type of the convulsions leading to muscular rigor in primary thermal shock. Certain animals had typical, severe, clonic spasms characterized by extensor rigidity. Some animals were thrown into a single, violent, tonic convulsion and remained in this condition. When rats were immersed for 1 second at 90° C., the convulsive movements frequently began with twitching and then vigorous shaking or shivering of the body. The typical clonic convulsion was followed by tetanic spasms of extensor rigidity of the entire body that preceded the death of the animal. Some animals experienced dyspnea and gasped for air during the time just prior to the clonic convulsion. Respirations were at first rapid, 150 to 200 per minute, and either gradually or suddenly became progressively slower until they reached 15 to 35 per minute.

The short time-exposures, by dipping the animals (except the head) for 1 to 10 seconds in water 75 to 90° C., were insufficient for the direct effect of heat to be transmitted to muscle by conduction. Some heat may have been transmitted through the blood stream. But when the skin was in contact with water at a temperature of 75° to 90° C., the



underlying gastrocnemius muscle in 24 animals had a temperature that varied from  $37.8^{\circ}$  to  $42.4^{\circ}$  C. The muscle rigor, therefore, was not the direct effect of heat on muscle producing heat rigor by protein coagulation. The clonic and tonic convulsions or cramps of the muscles were produced by reflex nervous action on the neuromuscular apparatus, arising from the initial sensory impulses generated by the caloric stimuli on the skin surface, reflected from the spinal cord through the motor innervation. The violent motor impulses transmitted to the neuromuscular apparatus were coincident in time with a massive transmission of axonic material into the end-plate and a torrential flow of the hypersecreted nervous substance away from the end-plate out into and between the muscle fibers. There was a corresponding and coincidental decrease in the diameters of the axons of the medullated branches of the sciatic nerve to the gastrocnemius muscle.

At autopsy the subcutaneous vessels were found enlarged, hyperemic, and densely packed with clumps of coagulated red blood cells. The intramuscular capillaries and venules, likewise, were enlarged in many places and in a condition of active hyperemia in those animals that were in thermal shock for 1 to 8 hours. Perivascular edema and packing of the minute vessels of the muscle with red blood cells were late manifestations of shock by heat. The muscles of those animals dissected within 10 to 30 seconds after the primary heat stimulus to the skin had the explosive pathologic changes in the motor end-plates, and not in the capillary bed of the muscles. The muscles in rigor produced by the reflex action of heat frequently did not respond to either direct or indirect mechanical or electric stimulation, but there was individual variation in the response. Many muscles in strong tonic contraction or tetanic spasm induced by the reflex action of heat did not manifest irritable retraction when transversely severed. Such muscles frequently had extensive axonorrhea or a discharge of the axonic nervous substance in the muscle. Many of the spastic muscle fibers were disorganized and in a condition of acute granular, hyaline, or Zenker's degeneration. During the late stages of thermal shock the muscles were frequently in a condition of flaccid paralysis. Such muscles had a dissolution of many of the motor end-plates and the nerve fibers terminating in them. There was a loss of the gold-staining substance in the motor end-plates that extended progressively in a centripetal direction in the epilemmal axons. This resulted in a local loss of many motor innervations of muscle fibers at the peripheral myoneural junction, and flaccid paralysis or muscle weakness. There was good correlation between abnormal structure and function of the neuromuscular appara-

tus. In the early stages of spastic palsy reflexly induced by heat, there was extensive secretion of nervous substance manifested by the gold-staining axonic material discharged into the muscle. In the later stages of thermal shock the animals were frequently in a condition of flaccid palsy. Many muscles from such animals had a structural loss of innervation at the junction between nerve and muscle. The pathologic function manifested by the skeletal muscle, therefore, was highly variable and was dependent upon the strength and duration of the stimulus, upon the time interval after onset of the stimulus, and upon the individual resistance of the animals to thermal shock.

Muscles reflexly manifesting a state of tonic spasm, rigor, or cramp due to the application of heat superficially applied to the end-organs of sensory nerves in the skin, presented two important problems: (1) Was the tonic spasm of muscle, induced by the nervous reflex to heat applied to the skin, caused solely by the transmission of immaterial trains of waves of electric energy constituting the nervous impulse to muscle? If so, morphologic methods are impotent to answer this question except by indirect evidence detected in alterations of the structure of the cross striations.<sup>7</sup> (2) Was the tonic spasm of muscle, induced by the nervous reflex to heat applied to the skin, associated in time with the transmission of a real material substance from nerve to muscle? If so, then morphologic methods are competent to reveal histologically this nervous substance discharged from nerve endings into muscle. The morphologic evidence herein presented demonstrates conclusively that there is a substantial transfer of nervous material from the nerve ending to muscle associated with functional activity.

The gross manifestations following shock produced by heat were comparable to those previously described for the viscera. The blood vessels of the gastrointestinal tract were pale, collapsed, and bloodless. The liver had blood vessels packed with red blood cells. Upon sectioning the liver, there was little or no bleeding. The spleen was retracted and failed to bleed upon sectioning, soon after trauma by heat. The kidneys and lungs had active hyperemia and vessels packed with red blood cells. These vascular changes became prominent during the late stages. Muscles were more red than normal ones and they were more friable and softer to teasing. The accumulation of metabolites evidently changed the consistency and elasticity of the fibers. Some paralyzed muscles failed to respond to transection of the spinal cord within 60 seconds after thermal trauma, as well as to direct mechanical cutting, and to direct and indirect stimulation by the galvanic and faradic currents. The rectus abdominis muscle demonstrated well the reflex

stimulation due to thermal shock to the skin by increased visibility of its contours: the lineae alba, semilunaris, and inscriptions.

There was manifested the so-called bloody tears or chromodachryorrhea in 10 per cent of the rats at various time-intervals after immersion at 75° C. for 10 seconds. This biologic response has been considered the effect of increased amounts of acetylcholine or dacryorrhetin in the circulating blood stream.

## II. The Pleomorphism of the Normal Motor End-Plates Compared with that Produced by Magnesium Sulfate and Strychnine

At the entrance of the axis cylinder of the medullated nerve fiber into the muscle fiber, the sheath of Schwann joins with the sarcolemma and the nerve loses its medullary sheath, but the axis cylinder passes into the muscle. The axon, upon entering the muscle fiber, divides dichotomously into a number of ramifications. The nerve plates (*terminaisons en plaque*) have two parts: (1) the ramifications of the axis cylinder, and (2) the variable amount of finely granular substance or sole plate of Kühne. This granular sole is abundant in the dark, retracted endings and is diminished in amount or completely depleted in the light, expanded plates. The axons of the terminal plate divide into many branches resulting in a variety of shapes. There are irregular dilatations and constrictions of the branches, like a string of beads. In the plate-like endings these ramifications are immediately under the sarcolemma. The granular sole is or is not in immediate continuity with the axonic divisions. The nuclei were divided by Ranvier<sup>8</sup> into three groups: (1) nuclei of the granular substance (*noyaux fondamentaux*); (2) nuclei belonging to the ramifications of the nerve (*noyaux de l'arborisation*); and (3) nuclei of the sheath of Schwann covering the terminal axons (*noyaux vaginaux*). It was impossible by morphologic criteria or differential staining to determine whether these nuclei are muscular or nervous in origin. The presumptive evidence was strong that the nuclei of the sole of Kühne were clusters of nuclei of the muscle fiber.

Where the epilemmal axon becomes continuous with the terminal plate-like expansion it divides and extends with further ramification, and thereby greatly increases its secretory surface area. The granular sole of Kühne is formed by the secretion granules of the discharging tips of the divisions of the axon. There is a direct transformation of the terminal axons in muscle into the granular sole of Kühne. The granules are poured out by the axons in arborescent arrangement and become incorporated into the myoplasm of the muscle fiber. Before

1874, Frey<sup>9</sup> (p. 323) made the following pertinent remarks, which have been ignored in recent times: "Now, if, as would appear to be the case, the distribution of the nerve fibre be confined to the immediate mass of the terminal plate, the extremities of the muscle fibre must remain without nervous supply, in that the former is set into the latter at about its middle. But the fleshy matter manifests contractility at the extremities also!"

The intramuscular axons, by progressive diffusion of the secreted granules, become merged throughout the fleshy mass of the muscle fiber. There is, therefore, a real anatomic blending of nerve and muscle by the periodic discharge of nervous material into the myoplasm. By the secretory mechanism of the motor end-plates there is anatomic and chemical continuity, and not discontinuity, as now taught, between nerve and muscle. There is a real, substantial fusion of nerve and muscle through the products of secretion of the nerve endings into muscle. There is, therefore, no constant line of demarcation between nerve and muscle. A search for it is like an attempt to discover a constant location where fresh water ends and salt water begins in the confluence between the Columbia River and the Pacific Ocean. This change depends upon many conditions, *viz.*, rate and quantity of flow, tide, and rate of diffusion.

The concept of Jordan and Speidel<sup>10</sup> that muscle has a structure composed of static membranes called sarcomeres, fixed in number, was an old one which had befuddled investigators of muscle structure for over a century. The changeable form of both motor end-plates and related cross striations invalidated any concept of constancy of structure, or fixation in number, of sarcomeres. The structure of the neuromuscular apparatus was highly variable corresponding to the periodic discharges of nervous substances into muscle. With each discharge the nervous substances blended with the myoplasm and there were concomitant changes of muscle structure as well as of the motor end-plates. The periodic discharges of nervous substances into muscle constituted one factor that determined the variation of internal structure of muscle.

Cannon<sup>11</sup> asked what standard we used to control our experiments in order to evaluate the normal from the pathologic changes in the motor end-plates. This was a pertinent question for the reason that characteristic changes were obtained by different local and general anesthetic agents, states of nutrition, and mechanical means employed prior to excising the muscle for microscopic study. The so-called normal had wide limits of variation. The motor end-plates quickly dis-

appeared during rigor mortis. Because of the normal sensitivity and pleomorphism of the motor end-plates, the problem was comparable to the study of the various complex forms of the waves that compose breakers along a diversified coastline. In this study we designated as standard or relatively normal (Figs. 1, 6, 23, 24, 25, and 27 to 32) those variations of form observed in the neuromuscular apparatus from muscles excised 15 minutes after the intraperitoneal injection of nembutal, 4 mg. per kg.

The relatively normal trees (Figs. 1 and 6) of motor end-plates have 10 to 20 per cent of the motor end-plates in a state of retraction and 80 to 90 per cent in a state of expansion in a differential count of 5,000 motor nerve endings, in 20 gastrocnemius muscles excised from 10 rats. The normal retracted endings surrounded by the granular sole varied from 20 to 40  $\mu$  in length. The expanded end-plates with a decreased quantity of the granular sole varied from 40 to 70  $\mu$  in length.

The cross striations were finer, closer together, and increased in number in relation to the expanded rather than to the retracted motor end-plates. If the motor end-plates did not influence the cross striations of muscle (and these cross striations are constant structures according to Jordan's<sup>12</sup> "sine qua non" for identification) the following questions are pertinent:

1. If the sarcomeres are constant in number in relation to each motor end-plate and in each muscle fiber, why do the expanded plates have finer striations, and more of the closely spaced striations, than are related to the retracted end-plates?

2. If the sarcomeres are constant in number in relation to each motor end-plate, would one not expect the striations to be spread farther apart in relation to the expanded plates and to lie closer together in relation to the retracted plates? The reverse relationship, however, was found.

3. If the sarcomeres are constant in number in a muscle fiber, how are they maintained when the discharge of nerve substance in muscle blends with the myoplasm?

4. Why are the cross striations frequently absent in relation to some motor end-plates?

There was, therefore, sufficient reason for the inclusion of those changes of end-plates excised from muscles in a state of flaccid paralysis 2 hours after the intraperitoneal injection of magnesium sulfate (Fig. 4), 2 gm. per kg. The retracted end-plates composed 25 to 35 per cent of the total of 5,000 end-plates counted in 20 gastrocnemius muscles from 10 animals. This proportion of retracted motor end-

plates (Fig. 4) was in contrast to that (Fig. 5) of expanded end-plates in the rigorous gastrocnemius muscle excised during an initial convulsion 10 minutes after the intraperitoneal injection of 0.5 cc. of 1:1000 strychnine sulfate. The expanded end-plates, with a diminution or complete depletion of the granular sole of Kühne, composed 95 to 98 per cent of 5,000 end-plates counted in 20 gastrocnemius muscles from 10 animals. There were numerous pseudopod-like, elongated branches in the motor end-plates during the early stage of expansion caused by strychnine.

This neoformative influence upon the ramifications and increase of surface area by ameboid expansion of the motor end-plates favored the secretory discharge of a fine spray of neuronc granules into muscle during the early period following strychnine stimulation. The chemical effect of strychnine stimulation had a morphologic expression in the structural changes of the motor end-plates and cross striations in the skeletal muscle fibers. The clear-cut photographic evidence of pleomorphism of the neuromuscular apparatus after nembutal, magnesium sulfate, or strychnine sulfate served as relatively normal and abnormal variations of changes of structure and as a base line of reference for evaluating the deviations produced by thermal shock.

The dark, anisotropic cross striations were coarser, and were separated by wider, light, isotropic bands in the flaccid muscle (Fig. 4) excised 2 hours after the injection of magnesium sulfate, than were those after the use of strychnine (Fig. 5). The latter were finer and more closely spaced than the former. This change of pattern was not due to the simple mechanical approximation and remotion of a constant number of sarcomeres in a "shuttle-like shift," like the opening and closing of the constant folds of an accordion. These changes in the cross striations were the structural expressions of changes in mechanical energy and chemical composition and concentration which are part of the ceaseless reactions of metabolism that underlie the variable tonicity, heat production, and mechanical activity of the muscular furnaces and internal combustion engines of motion of the living organism.

### *III. The Histopathology of the Neuromuscular Apparatus in Thermal Shock*

1. *Achrysophilia and Hypochrysophilia of Motor End-Plates.* Muscles excised from rats 2 hours or more after immersion in water, except the head and neck, 75° C. for 10 seconds, had the majority of the motor end-plates (3,765 of 5,000 counted) completely liquefied (Figs.

3, 49, 68, 69, 70, 72, and 74). This final result leading to explosive disappearance of the motor endings was likewise related in many places (Figs. 68 to 70, and 74) to an acute granular degeneration and hyalinization of the cross striations of the muscle fiber. These changes of the hypolemmal axons of the motor end-plates were likewise accompanied by different degrees of depletion ending in complete exhaustion (Fig. 74), achrysophilia, and hypochrysophilia, of the substance with an affinity for gold in the epilemmal axons.

The liquefaction of the motor endings resulted in structural loss of innervation at the neural junction. Progressive hypochrysophilia to achrysophilia were those final conditions of exhaustion in which the axonic materials of the motor end-plate were discharged and dispersed in the myoplasm.

In some locations (Figs. 68 to 70), where the motor end-plates had disappeared, the clusters of dark, rounded, pyknotic nuclei, 6 to 15 in number, were clearly revealed by counterstaining with hemalum after gold impregnation. These nuclei of the depleted sole of Kühne appeared to belong to the myoplasm. They frequently were made visible without a counterstain when lactic acid was injected locally in the muscle. Lactic acid caused a rapid disappearance of the motor end-plates and paralysis due to the peripheral local loss of innervation in the muscle.

2. *Hyperchrysophilia of the Motor End-Plates.* The first morphologic result of heat applied to the skin was reflexly produced by an augmentation and overflow of the quantity of substance in the motor end-plates which had an affinity for gold (Figs. 2, 7, and 50 to 62). This increased blackening of the motor end-plates was not due to over-staining. The normal and abnormal muscles were run through the same technical procedures at the same time. This increased affinity of the end-plates for gold, due to the transmission of increased amounts of substances from the epilemmal into the hypolemmal axons, was accompanied by variations in the structure of the axons proximad to the plate. There were variations in the size of the swellings and constrictions in the moniliform patterns of the epilemmal axons. The changes in structure observed were compatible with increased conduction distally of nerve substances into the terminal axons of the motor end-plates.

3. *Axonorrhea of the Motor End-Plates.* The second result of the reflex activity of heat applied to the skin was an abnormal discharge and projection (Figs. 8 to 22, and 50 to 67) of the nervous axonic substances into the myoplasm of the muscle fiber.

The best time to observe this short-lived phase in the secretory cycle

of the motor end-plates was within 10 to 30 seconds after the rat was immersed in water, except the head and neck, for 1 to 10 seconds at 90° C. The gastrocnemius muscle was in a reflex state of spasm or rigor and had a muscle temperature of 37.8° to 42.4° C. The initial effect of heat applied to the skin for short durations on the form of the motor end-plates was via the nervous reflex arc and not by direct conduction from skin to muscle. The muscle was excised immediately and processed at once for gold impregnation. This period in motor end-plate discharge was both tenuous and ephemeral. The quantity of discharge was more copious after reflex action due to heat stimulation (Figs. 2, 7, and 50 to 62) of sensory nerves in the skin, however, than it was in the normal (Figs. 1, 6, 23, 24, 25, 27 to 38, 71 and 73) controls.

This discharged axonic material in the muscle fiber was either densely opaque with gold impregnation or cross-striated. Its cross striations were or were not in alignment with those of the muscle fiber. There were various stages in the projection, diffusion, and dissolution of the neuronic substances secreted into the myoplasm of the muscle fiber. The cross striations of the muscle offered no obstacle to the projection and diffusion of the axonic substances discharged from the motor end-plates. These cross striations, therefore, were not rigid membranes or hard and fast partitions. The easy projection of axonic substances and streamlining of the cross striations were morphologic evidences that render untenable the theory that the muscle fiber is divided into a constant number of compartments or sarcomeres by rigid partitions. There was good correlation of abnormal muscle structure and function by the morphologic presence of the nervous discharge of inclusion masses of Kühne's granules into the myoplasm and the spasm of muscle reflexly induced by heat.

The discharged nervous secretion into the myoplasm had an increased affinity (hyperchrysophilia) for gold and was in continuous relationship with the motor end-plate or projected as discrete masses throughout the muscle fiber. The arrangement of the discharged secretion in juxtaposition to the motor end-plate was unipolar (Figs. 50 to 55); bipolar (Figs. 58 and 59), or multipolar (Figs. 8, 10, 61, and 62). The form of the nervous substance secreted into the myoplasm was likewise variable, namely: (1) as discrete granules (Figs. 12 and 63); (2) as vacuoles (Fig. 64); (3) as short or long fusiform and irregular masses (Figs. 65 to 67); and (4) as short or long, Indian-club or arrow-shaped projections (Figs. 10 and 11). The axonic masses projected away from the nerve terminal were usually within the myoplasm but some were found external to the sarcolemma of the muscle



fiber. In such conditions the motor end-plates were missing and the epilemmal axons terminated external to the sarcolemma of the muscle fiber.

The axonic material descended from the epilemmal axons into the hypolemmal axons and discharged between and away from the branches of the end-plate. This discharged secretion formed either a small (Fig. 40) or a large hub (Fig. 41) in the end-plate. This central mass of Kühne's granules formed, at times, a ring with a thick rim (Fig. 42) from which the mass or masses of discharged nervous substance extended into the myoplasm. The hypolemmal axons of the motor end-plate became attenuated as they were transformed into the granules of Kühne (Figs. 44, 47, and 48). This transformation of axons into granules was well advanced in some places (Figs. 48 and 49). The periodic internal structure of the muscle was replaced by a diffuse arrangement of granules (Fig. 49).

These masses of discharged nervous secretion into the muscle underwent progressive decrease in size and incorporation into the colloidal substance of the myoplasm of the muscle fiber. It was clearly evident, therefore, that there was no fixed boundary or line of demarcation between the junction of nerve and muscle. The substantial secretion from the motor nerve ending became incorporated into the explosive mixture that forms the active muscle.

There were many examples of so-called ultraterminal branches (Figs. 18 to 22) of the motor end-plates in the very early periods after shock due to scalding. The term "ultraterminal" was applied to an unmyelinated branch which arises from the ramifications of the axonic terminals of the motor end-plates. These ultraterminal branches terminated in rudimentary swellings in the same or in neighboring muscle fibers. Both types of terminals have been observed. They were the product of overstimulation due to multiple causes. They were frequently found in the very early stages of primary shock due to scalding of the skin of rats. They represented an early stage in the explosive projection of axonic secretory material from the motor end-plates due to reflex overstimulation. They were found under various conditions of anoxia. At another instant of time the projected material became disconnected from its point of origin in the axonic branches of the motor end-plates. These projected secretory masses of axonic material then became discrete bodies of inclusions in the myoplasm of the muscle fiber. This stage of axonic inclusion masses in muscle was a very temporary one. The nervous material discharged into muscle soon underwent dissolution. The accumulation of acids and other metabo-

lites in muscle due to overstimulation and anoxia causes a delay in the dissolution of acetylcholine deposited physiologically at the synapses, according to Gesell and co-workers.<sup>13</sup>

The end-plates showing these sequences of changes of their hypolemmal axons following the reflex activity of scalding the skin—(1) hyperchrysophilia, (2) axonorrhea, (3) hypochrysophilia, (4) achrysophilia, and (5) centripetal degeneration or depletion of the epilemmal axons—were intermingled with relatively normal end-plates. Large quantities of muscle tissue were teased in order to reveal the relative proportion of the pathologic changes of the end-plates. Certain axons of a motor tree manifested excessive pathologic changes; others remained relatively normal in the same or neighboring trees of innervation.

4. *The Centripetal Depletion of the Epilemmal Axons.* The gastrocnemius muscle (Fig. 74) was excised 2 hours after the entire body of the rat, except the head and neck, had been immersed for 10 seconds in water at 75° C. There was complete disappearance of the motor end-plates (achrysophilia) and diminution of the capacity of the granular epilemmal axons for gold impregnation (hypochrysophilia). The depletion of these axons extended in a centripetal direction and away from the site of the granules that occupied the previous site of the motor end-plates. Thermal shock, therefore, produced a structural exhaustion of both the epilemmal axons, that extended proximally for variable distances, and hypolemmal axons of the motor end-plates.

5. *The Pathologic Changes in the Muscle Fibers.* The time of appearance of spastic and flaccid paralysis depended upon individual resistance of the rats, upon the temperature of the water, and duration of immersion. The time interval after traumatic scalding was likewise a factor in the degree of intensity of pathologic changes in the muscle fibers. During the early period after scalding the cross striations in many places were replaced diffusely by granules (Figs. 49 and 74) arranged especially in close relationship to the motor end-plates. The muscles, whose irritability was reduced or lost, had Zenker's waxy degeneration localized in some fibers. There was increased visibility of pyknotic nuclei in certain muscle fibers. There was a loss of the differential types of fibers in the majority of the muscles examined microscopically. There was little alteration of the intramuscular capillaries during the very early periods, 10 to 60 seconds after scalding. These capillaries were dilated and packed with clumps of red blood cells in many places within the muscles 2 hours after the heat stimulus (75° C. for 10 seconds) was applied to the skin. During the early stages the lesions of the muscles were dominant in the neuromuscular apparatus.

During the late stage hyperemia, perivascular edema, and leukocytic infiltration were prominent findings.

By counterstaining with hemalum there were found clusters of dark, pyknotic, rounded nuclei, 6 to 15 (Figs. 68 to 70) in number, that were related to the sole plate. These nuclei appeared to be modified muscle nuclei. Within the cluster of nuclei of the depleted motor end-plates there was granulation, in some places, of the cross striations of the muscle fiber. These morphologic changes were comparable to those produced by the injection of lactic acid locally in the zone of innervation of the muscle. The epilemmal axons frequently ended in bulbous expansions (Figs. 68 to 70) and had complete depletion of their motor end-plates. The function of these sole plate nuclei is under investigation.

### DISCUSSION

The prevailing static concept of the relation of the motor end-plate to the striped muscle fiber is one of a mechanical penetration and crude apposition, of discreteness and separateness of two vitally united structures. This may be one effect of the cell theory based upon the concept that the muscle cell and nervous cell are completely discrete structures in all of their parts. The dynamic interrelationship of muscle and nerve is an acceptable point of view to account for the periodic discharge of nervous substances into muscle. This results in a blending of the neuromyoplasm in a functional dynamic unit.

The initial mixture of nerve and muscle working-substances quickly forms a compound of complex colloidal neuromyoplasm. The nervous discharge may be a fine spray or a flood of secreted substances. A complex explosive mixture of the nerve and muscle substances, like a mixture of mercury fulminate and black powder, becomes thoroughly commingled until the nerve substance is nondetectable as such in the neuromyoplasm. The periodic mild and strong chemical reactions produced by the explosive mixture in an elongated capillary strand of nerve and muscle substances, confined in one fiber, are reflected in either regular and ordered cross striations or irregular and completely disordered arrangements of the internal structure of the cross striations.

The cross striations of living, nerve-intact muscle, therefore, are the mechanical products and structural expressions of capillary chemism enclosed in an elongated capillary system of neuromyoplasm. During life there is a ceaseless replacement of one system of cross striations by another. There are variations of amplitude and of combinations of one system of periodic bands with another. There may be mathematical additions and subtractions of constructive and destructive interfer-

ences, respectively, of these various systems of longitudinal pressure waves, which are expressed in the variable internal structure of skeletal muscle. Any concept that attempts to simplify the internal structure of muscle, for descriptive purposes, by postulating constancy of sarcomeres and alphabetical symbols, does not conform to easily reproducible facts obtained by experimental morphology. This static concept of structure has hindered the correlation of the anatomy and physiology of nerve and muscle for over a century.

The cross striations are the structural expression of periodic pressure waves composed of alternate zones of condensation and rarefaction that accompany the periodic explosive chemical changes in the capillary skeletal muscle fiber. This fiber, therefore, is dual in nature: (1) Its chemical composition undergoes reversible and irreversible quantitative and qualitative changes that are periodic or aperiodic; and (2) the chemical reactions confined in capillary spaces are accompanied by the physical explosive energy of internal compression waves. These align the protoplasmic colloidal mixture of the skeletal neuromuscular fiber into longitudinal waves of alternate zones of compression and rarefaction. This spectrum of internal compression may be used to estimate both the intensity and speed of chemical reactions and of the physical internal pressure which is synchronous with the explosive chemical reaction. The revelation of the secret of the transformation of the chemical energy of muscle into mechanical work lies in the field of morphology correlated with that of chemistry, physiology, and pathology. The attached myoneural capillary fiber gives direction to the explosive action of the muscle protoplasm roughly analogous to the direction given by the gun barrel to the explosive action of its gun powder, but the gun barrel, the skeletal muscle fiber, is closed at both ends.

Thermal shock produces pyknotic changes of the nuclei of the granular sole of Kühne. The functions of these nuclei are unknown. Do they contribute to the synthesis of the hypothetical transmitter substance—acetylcholine? Do they aid in the manufacture of choline esterase? The combined methods of morphology and that used by Stoerk and Morpeth<sup>14</sup> in the estimation of choline esterase activity in skeletal muscle may aid in finding the answers to these questions.

The so-called bloody tears or chromodacryorrhea was considered by Tashiro and Stix<sup>15</sup> as the biologic effect of acetylcholine or dacryorrhetin. Dacryorrhetin was found in an extract of muscle and considered a product of muscle metabolism. There appeared to be a correlation between the abnormal changes in the harderian glands of the lacrymal

apparatus and those of the neuromuscular apparatus in thermal shock in the rats. Additional experimental work was suggested by this interrelationship.

From a very early period, the nature of the terminals of nerve in striped muscle has occupied the attention of anatomists and physiologists. Before the use of the microscope, conjecture alone was possible. It was supposed that the terminal twigs of a nerve broke up into finer and finer branches which became finally fused with the muscle. The nerve fiber was considered a conduit for the transmission of a vital spirit which in some manner was delivered to the organ activated by nerves.

That the nerve fuses and commingles with muscle by the discharge of some ephemeral and tenuous substance through the secretory mechanism of the motor end-plates is nearer the truth than anatomists have realized since the introduction of the microscope. The termination of nerve in muscle is one of a fusion, a commingling of their respective substances and not one of cellular separation by partitions, like watertight compartments. There is thus established a morphologic and functional continuity and dynamic unity of the organization of nerve and muscle.

The intact living muscle fiber with its blood supply and attachments should be looked upon as a morphologic and functional whole composed of neuromyoplasm. This applies to both red and white muscle fibers. This unit may have periodic changes in its internal structure of longitudinal and cross striations corresponding to changes of function and nutrition. The inconstant longitudinal striations are evanescent mechanical products of streamlining produced by metabolism and the mechanical tension and pressure due to changes of function and nutrition. The specious division of the muscle fiber into stable contractile myofibrils and constant, intermediate, nutritional sarcoplasm does not conform to the facts. Some muscle fibers have well defined longitudinal striations and granular sarcoplasm; other neighboring fibers in the same muscle do not possess longitudinal myofibrils. These morphologic differences are correlated with different levels of metabolism and functional activity. One type readily transforms into the other, but both stages perform their tonic, thermal, and contractile functions. The muscle fiber is activated as a whole throughout its neuromyoplasm. No adequate evidence has been presented to support the assumption that the ephemeral, longitudinal myofibrils of the muscle fiber are the only structures capable of contraction in the

muscle fiber. The neuromyoplasm of the skeletal muscle fiber, with or without a sarcolemma, functions as a whole; it may or may not be cross striated; it may or may not be longitudinally striated into ephemeral myofibrils.

The histologic characteristics of the dorsal deep component of the sternocleidomastoid muscle in the rat are those of a specific red muscle fiber. Its fibers are smaller in diameter on the average and more granular than the easily separated, ventral, superficial part of the muscle. The inclusion of pigmented granules of myohemoglobin evidently acts as a storage of oxygen for the intrinsic respiration and metabolism of the deep part of the muscle.

The red pigment of hemoglobin compounds is found in the larval blood-worms inhabiting the warm springs of the Yellowstone National Park. Brues<sup>16</sup> and Leitch<sup>17</sup> have studied experimentally the rôle of hemoglobin in the larval blood-worms of the dipterid insect, *Chironomus*. They came to the conclusion that the function of hemoglobin in the blood-worm and snail in thermal springs consists in making available, by the power of binding oxygen chemically, a quantity of oxygen sufficient for the needs of the animal at oxygen tensions so low that the necessary amount is not supplied by physical solution. There is a dearth of dissolved oxygen in thermal waters which renders respiration difficult for purely aquatic animals. Hemoglobin is characteristic of many mud-inhabiting invertebrates that live where oxygen is present in only very small amounts. The larvae of *Chironomus*, by the presence of hemoglobin, are thus able to live under relatively anaërobic conditions and, furthermore, an increased alkalinity serves in some way to increase the resistance of these animals, according to Packard,<sup>18</sup> to a lack of oxygen.

The presence of a muscle pigment of hemoglobin would give a local supply of oxygen within the muscle where there was a decrease of the availability of oxygen. Whether or not white muscle could be transformed into red muscle by elevations in temperature, much as Huggins and co-workers<sup>19</sup> have found that increase of temperature transforms yellow into red bone marrow, is a problem under investigation. Each red or white muscle fiber, respectively, functions as a unit and is composed of the chemical fusion of nerve and muscle substances to form the neuromyoplasm. The structural changes in both types of muscle fibers are correlated with different levels of functional activity. There are, however, physiologic differences between red and white muscle fibers that have not been completely correlated with structure.

## SUMMARY

1. Whole-mount specimens of the gastrocnemius muscle and the motor end-plates from 250 rats subjected to thermal shock produced by water immersion (except the head and neck) at  $75^{\circ}$  to  $90^{\circ}$  C. for 1 to 10 seconds, were studied by the gold and teasing method; the gastrocnemius muscles were excised from 20 rats 15 minutes after light nembutal anesthesia and used as controls. The muscles were excised from within 10 seconds to 3 hours after the thermal trauma. The muscles from the control series and the traumatic series were divided into groups and run through the gold technic together. Exact comparison of the quantity and quality of the gold impregnation in the relatively normal control series and in the traumatized series was made by this method.

2. There was individual variation in resistance of the rats to primary thermal shock. Some of the animals, immediately upon immersion, had severe clonic spasms and paroxysms, or convulsions that resulted in tonic extensor rigidity. Others manifested mild twitching at first, and then strong, shivering, fascicular movements of the muscles. After 1 to 3 hours many of the rats had flaccid paralysis of one or more extremities. There was variation in the transition from violent muscular movements and from a spastic to a flaccid state of the muscles, as well as in the degree and extent of loss of muscle motion. These functional changes were dependent upon: (1) individual resistance of the animals; (2) duration of immersion; (3) degree of temperature; and (4) time interval after onset of the violent heat trauma.

3. The temperature of the muscles varied from  $37.8^{\circ}$  to  $42.4^{\circ}$  C., immediately after immersion of 24 rats. There was very little direct conduction of heat, therefore, from the skin to the spastic skeletal muscle. It was concluded, therefore, that the initial and variable pathologic function of muscle in immediate response to cutaneous scalding was produced by violent impulses transmitted through the neuromuscular apparatus. Some of these muscles, during the early stage of heat shock, failed to respond to both direct and indirect electric stimulation. This likewise applied to certain flaccid muscles in the late stage of shock. There were variations of response of the muscles in both a generalized and localized manner.

4. The majority of the motor end-plates had disappeared in 75 per cent of the muscles examined within 1 to 3 hours. The ordinary sequence of histologic changes, intermingled with normal appearances, in the motor end-plates were as follows:

(a) Hyperchrysophilia or increased intensity of gold impregnation

due to increased quantity of axonic material transmitted to the motor end-plate.

(b) Axonorrhea or the abnormal discharge of the axonic material from the motor end-plates, increased in permeability, into the myoplasm of the muscle fiber. The substantial secretion from the motor end-plates was arranged in a unipolar, bipolar, and multipolar pattern. The pleomorphism of the discharged secretion was manifested as granules, vacuoles, short or long fusiform inclusion masses in the myoplasm, short or long Indian clubs, triangles, or irregular masses that had a greater affinity for gold than the myoplasm. The length of the axonic material secreted into muscle varied from 0.5 to 1000  $\mu$ . The non-medullated projection of axonic material still connected with the motor end-plate has been previously described as an "ultraterminal branch." Such projections were frequently found during the early primary effects due to reflex overstimulation by scalding the skin. The finding of large amounts of inclusion masses of the discharged nervous secretion in the myoplasm was synchronous with the manifestation of rigid spasticity of the muscles. These nervous inclusion masses in the myoplasm were either opaque or cross-striated.

(c) Hypochrysophilia and achrysophilia were evident as a progressive lack of gold impregnation finally leading to the complete absence of gold in the terminal of the nerve. This lack of gold impregnation was due to the complete absence of the terminal axonic structures. These had undergone granular transformation and dispersion in the myoplasm. The final stage was a complete structural exhaustion of the motor end-plates coincident in time with the flaccid paralysis. There was muscle denervation at the myoneural junction produced by complete structural exhaustion. This exhaustion was the result of the sudden axonic transformation into granules which composed the products of the acute hypersecretion into muscle induced by scalding the skin. The abnormal heat stimulus to the skin accelerated the drainage and depletion of the axonic fluid in motor nerves.

(d) The granular exhaustion of the epilemmal axons extended in a centripetal direction. The motor end-plates appeared to be vulnerable chemical fuses between the peripheral nerves and muscles.

5. There were acute changes in the myoplasm in addition to the great quantities of inclusion masses of nervous secretion: granular, hyaline or Zenker's degeneration, and pyknosis of nuclei.

6. During the early stages, 10 to 60 seconds after traumatic scalding of the skin, the dominant change in the muscle was in the neuromuscular apparatus. After 1 hour there were prominent vascular



changes: active hyperemia, packing of the vascular lumina with red blood cells, perivascular edema, and leukocytic infiltration. There was individual variation of the rats in manifesting this usual sequence of pathologic reaction to the abnormal motor impulses generated by the reflex response to burns.

7. Ten per cent of the rats subjected to immersion in water, 75° C. for 10 seconds, manifested the so-called bloody tears or chromodacryorrhea of the harderian glands. It has been established previously that this biologic response was produced by acetylcholine or by the breakdown products of muscle metabolism called dacryorrhetin. There appeared to be a correlation in the isolated changes in the neuromuscular apparatus and in the harderian gland in the rat.

8. The nerve-intact skeletal muscle fiber should be considered as a functional and morphologic unit composed of neuromyoplasm; it may or may not possess a sarcolemma; it may or may not have cross striations; it may or may not be longitudinally striated or subdivided into myofibrils. The variable cross striations are structural expressions of the changeable mechanical energy of explosive pressure that accompanies the capillary chemistry of metabolism. The ephemeral longitudinal striations are streamlining products of the periodic changes of metabolism and of the mechanical tension and pressure accompanying muscle function. The cross and longitudinal striations are changeable and not constant structures. The myofibril, therefore, cannot be considered the constant specific structure that subserves the contractile function. It is a mechanical, structural effect and not the definite morphologic cause or sole protoplasmic substratum underlying muscle contraction.

Gratitude is expressed to Dr. G. Kasten Tallmadge, Assistant Professor of Anatomy, for reading the manuscript; and for technical assistance in the teasing of muscle to: Miss Estelle Downer, Messrs. E. Socolof, R. Jeub, J. Hamel, C. Saribalis, J. Sweeney, J. Raggio, S. Davito, and to the members of the 1944-1945 freshman class in medicine.

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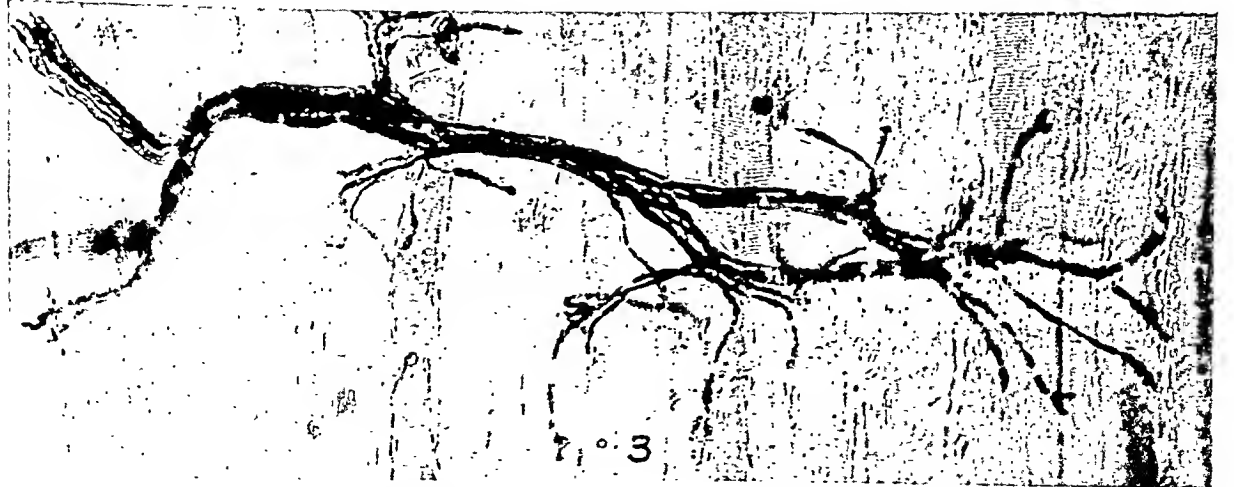
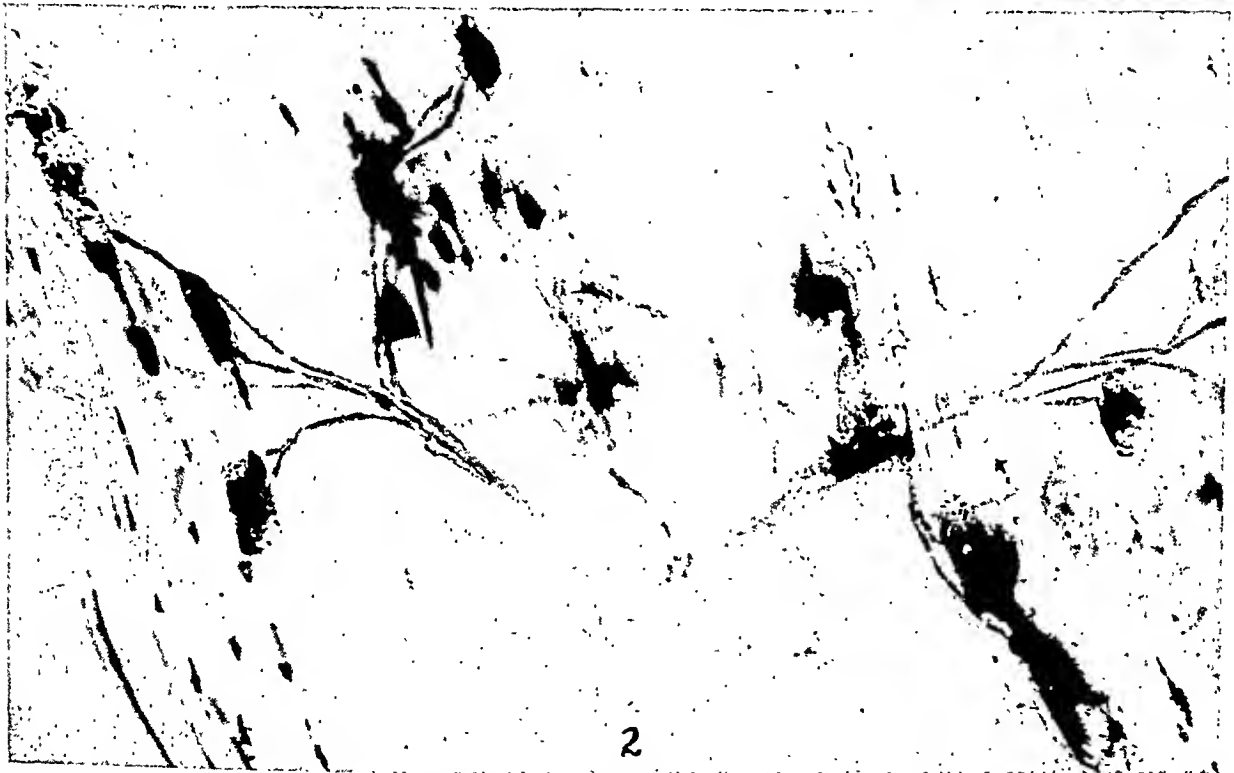
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### DESCRIPTION OF PLATES

The photomicrographs of Figures 1 to 71 are from teased whole muscle fibers (gastrocnemius muscle) and motor end-plates of the white rat (*Mus norvegicus*). These teased preparations of motor end-plates in skeletal muscle were previously prepared by the gold technic. The photographs were made as direct contact prints from the negatives which were exposed through the microscope and not subjected to subsequent enlargement. These photographs, therefore, may be readily compared with those of the the white rat and the chameleon previously published.<sup>6</sup> In the plates, "epa." designates the epilemmal axon; "hya." the hypolemmal axon; and "Kg." masses of extra-axonic Kühne's granules. There has been no retouching of either negatives or prints.

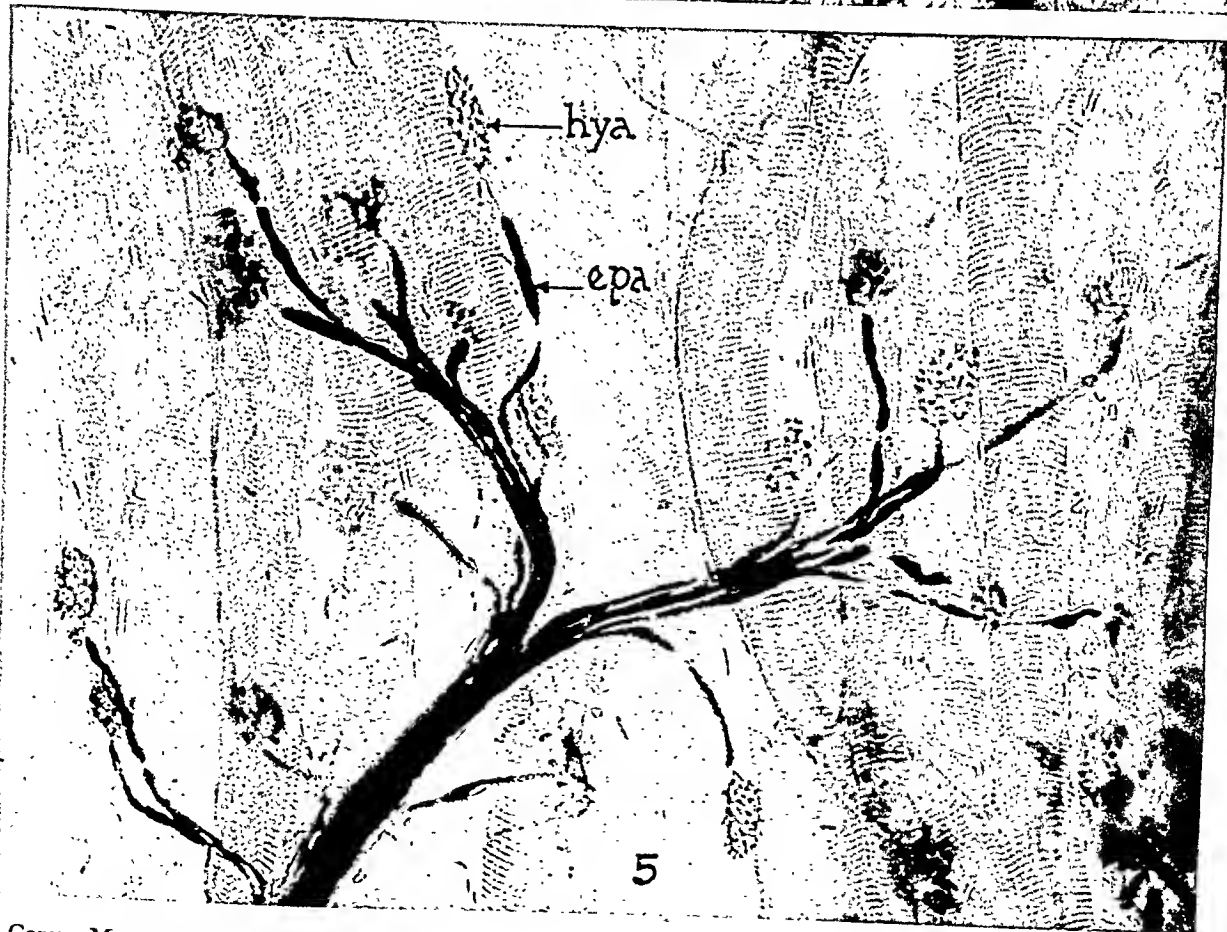
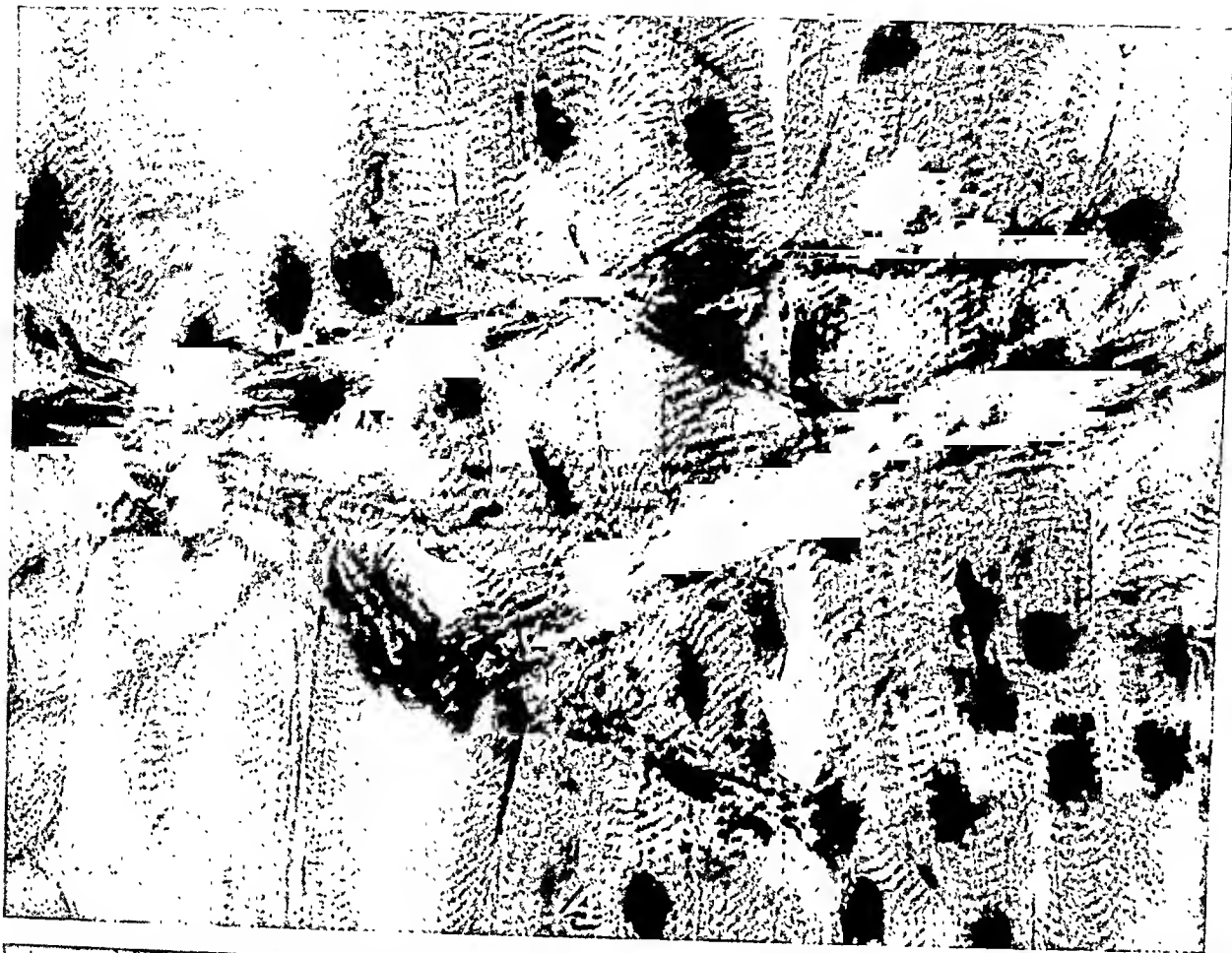
### PLATE 36

FIGS. 1 to 3. Sprays of axons of medullated nerve fibers and motor end-plates of the white rat (*Mus norvegicus*) in the relatively normal gastrocnemius muscle excised 15 minutes after the intraperitoneal injection of nembutal, 4 mg. per kg. (Fig. 1); muscle excised 30 seconds after immersion of rat at 90° C. for 10 seconds (Fig. 2); muscle excised 2 hours after immersion of rat at 75° C. for 10 seconds (Fig. 3). In the relatively normal muscle (Fig. 1) there is a variation in the type of muscle fibers and in the sizes of the motor end-plates. Some motor end-plates in the retracted condition are surrounded by a dense accumulation of Kühne's granules. The light, expanded end-plates have a diminution or complete absence of the surrounding granules of Kühne. The first morphologic effect of the heat stimulus applied to the skin was hyperchrysophilia (Fig. 2), increased intensity of gold impregnation of the enlarged motor end-plates. This was due to the increased accumulation of nervous material in the end-plate prior to discharge into the muscle. In some locations the discharged nervous secretion is evident in the muscle fiber. The epilemmal axons (Fig. 2) manifest beginning exhaustion of their substance. The complete dissolution of the motor end-plates (Fig. 3), and the centripetal depletion of the epilemmal axons of gold-impregnated substance are the last events in a whole series of changes that occur in the peripheral denervation of muscle at the myoneural junction subsequent to the shock stimulus produced by heat applied to the skin. The neuromuscular apparatus, therefore, manifests in thermal shock the following sequence of changes: (1) hyperchrysophilia of the enlarged end-plates; (2) axonorrhea, or abnormal discharge of nervous secretion into muscle from the abnormally permeable motor end-plates; (3) hypochrysophilia; (4) achrysophilia; and (5) depletion of the gold-impregnated material in the epilemmal axon in a centripetal direction. One clearly evident result of the reflex action of heat applied to the skin is the loss of the dark type of muscle fiber (Figs. 2 and 3) due to simultaneous contractions of the great majority of these fibers. The fractional contraction of normal muscle is structurally expressed by the presence of dark and light muscle fibers (Fig. 1).  $\times 150$ .



### PLATE 37

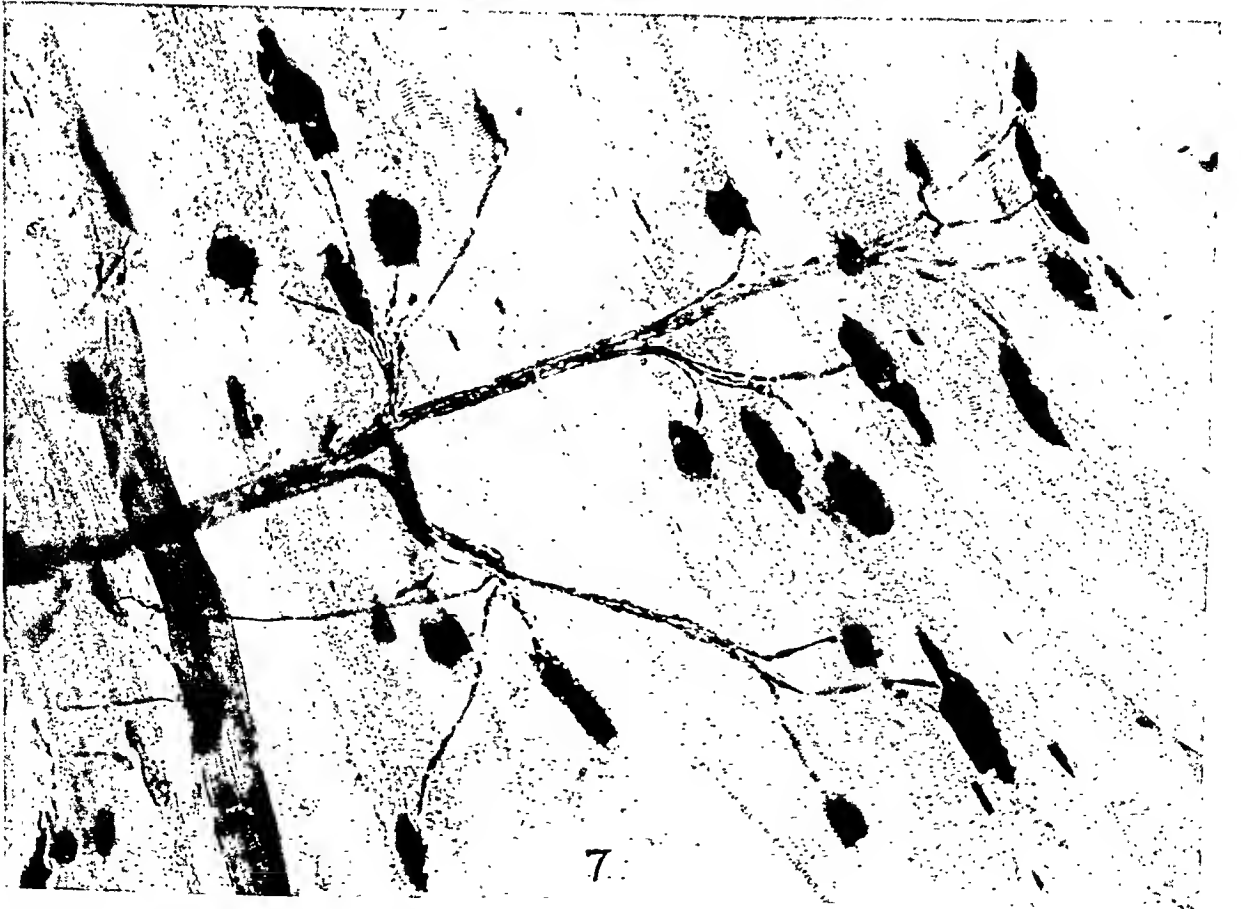
FIGS. 4 and 5. Sprays of axons of medullated nerve fibers and motor end-plates of the gastrocnemius muscle of the white rat, 4 hours subsequent to the intraperitoneal injection of magnesium sulfate, 2 gm. per kg. (Fig. 4), and 10 minutes subsequent to the subcutaneous injection of strychnine sulfate (Fig. 5). There is a great retraction of about 35 per cent of the motor end-plates in the gastrocnemius muscle after the injection of magnesium sulfate. The epilemmal axons are faintly impregnated with gold. The muscle fibers are narrow and the cross striations form coarse bands. The muscle is in a state of flaccid paralysis. Some muscles, in flaccid paralysis due to magnesium sulfate, failed to respond to indirect stimulation of the sciatic nerve. By direct stimulation the muscles responded feebly to electric stimulation. The above changes (Fig. 4) are to be contrasted with those produced by strychnine sulfate (Fig. 5). Ninety-five per cent of the endings are expanded, and in some places the hypolemmal axon is fragmented (Fig. 5) into discrete granules, which give the stippled appearance. The epilemmal axons are wide in some places and very narrow in others and are intensely impregnated with gold. About 95 per cent of the muscle fibers have fine, closely spaced cross striations. These changes of retraction and expansion of the motor end-plates and the configuration of the cross striations produced by chemical action are to be used as a base line of departure in the interpretation of the changes produced by thermal shock.  $\times 300$ .



## PLATE 38

FIGS. 6 and 7. Sprays of axons of medullated nerve fibers and motor end-plates of the gastrocnemius muscle of the white rat, in the relatively normal animal (Fig. 6), obtained 15 minutes after the intraperitoneal injection of nembutal. There is variation in the type of muscle fibers and in the sizes of the motor end-plates. Some motor end-plates in the retracted condition are surrounded by a dense accumulation of Kühne's granules. The light, expanded end-plates have a diminution or complete absence of the surrounding granules of Kühne. It is evident that the first morphologic effect of the heat stimulus of a 10-second skin exposure in water at 90° C. is a hyperchrysophilia of the motor end-plates (Fig. 7). The muscle was excised 10 seconds after immersion. An augmented discharge of masses of Kühne's granules from the end-plates is made evident by the intense impregnation of this nervous material by gold. There is a beginning depletion and beading of the epilemmal axons. This phase of the secretion of the nervous material into the muscle fiber is a very early and ephemeral one. It soon gives way to explosive projection, diffusion, and subsequent dissolution of this nervous material in the myoplasm of the muscle fiber.  $\times 150$ .





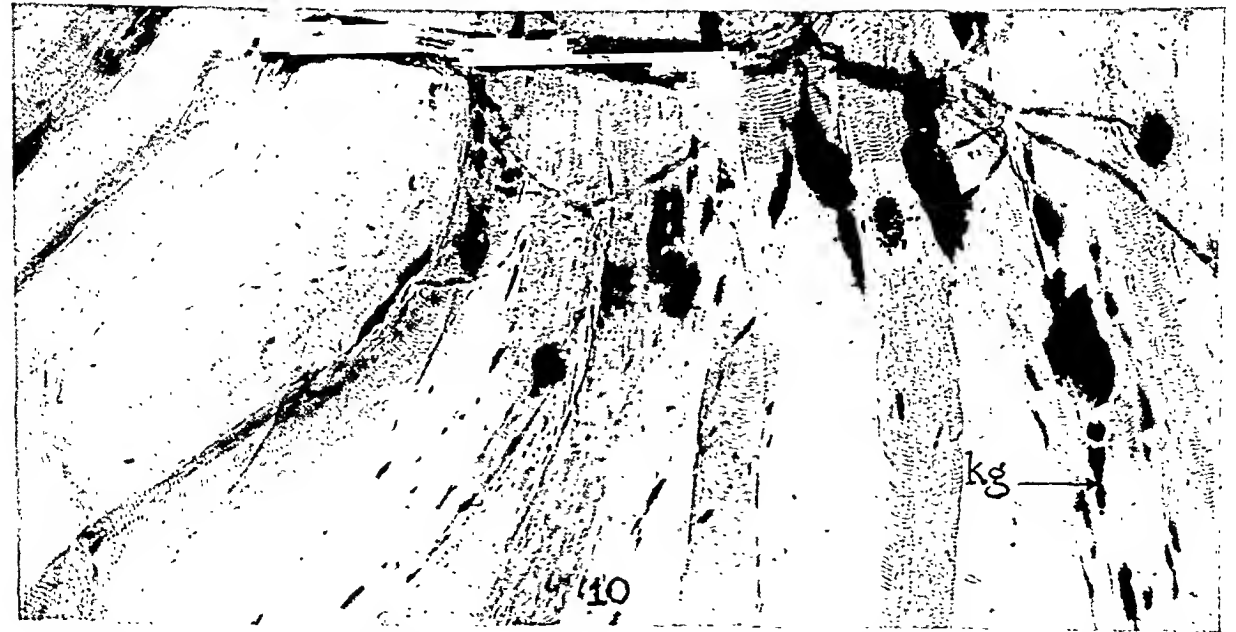
Carey, Massopust, Zeit, and Haushalter

Motor End-Plates in Thermal Shock



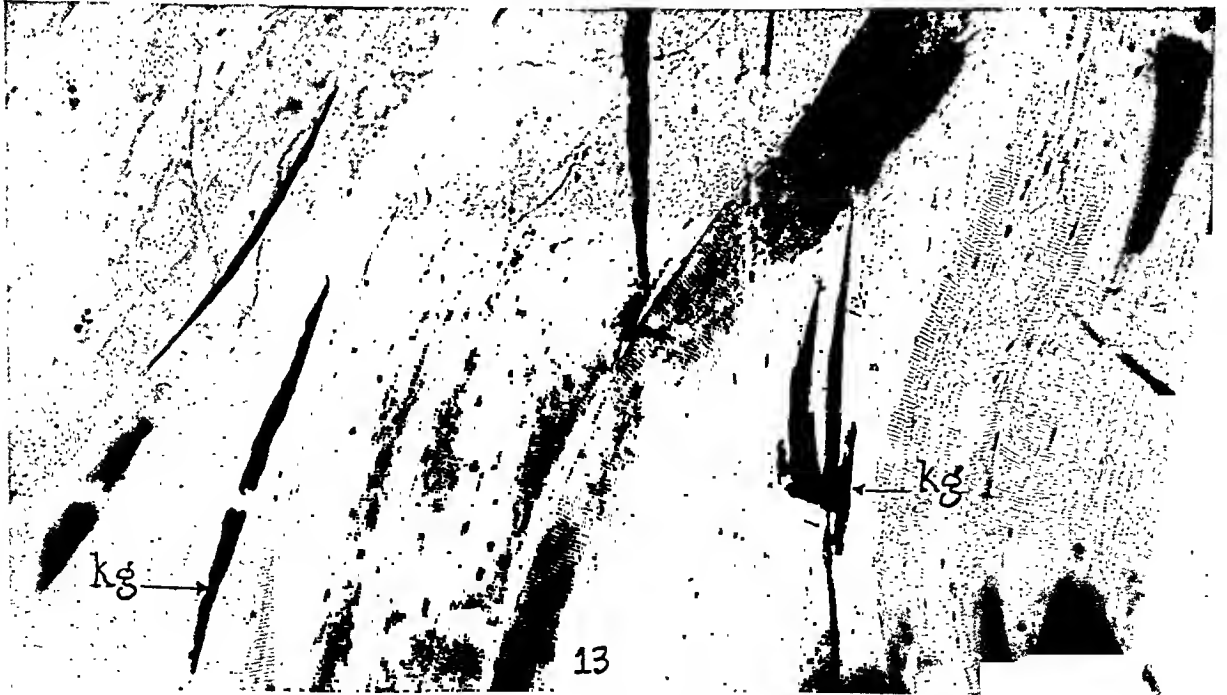
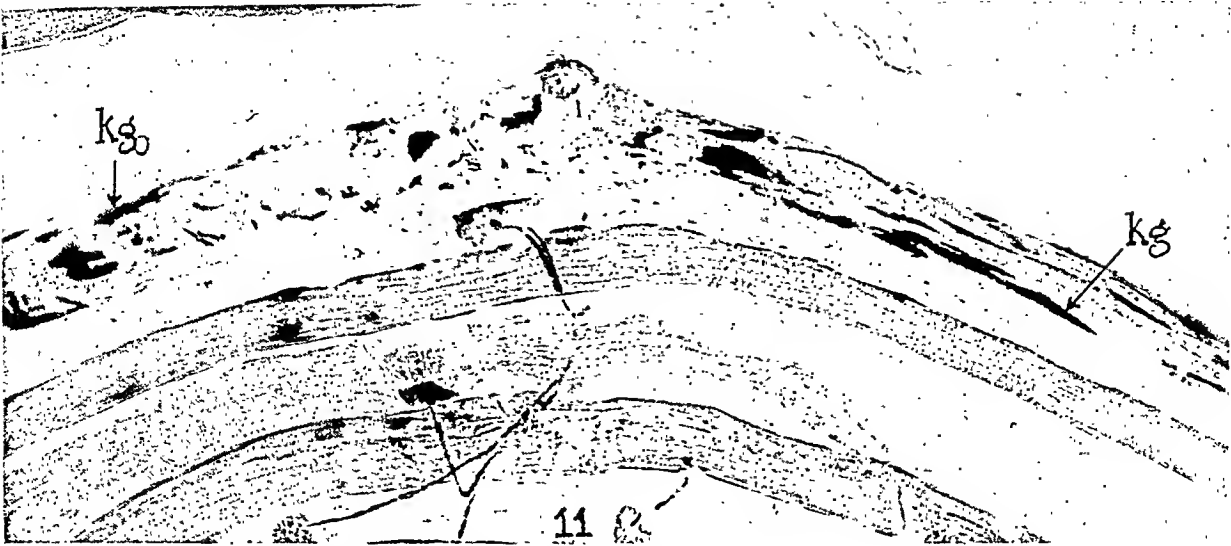
## PLATE 39

FIGS. 8 to 10. Sprays of axons of medullated nerve fibers and motor end-plates of the gastrocnemius muscle of the white rat, 30 seconds subsequent to the heat stimulus of a 10-second skin exposure in water at 90° C. The first phase of the neuromuscular reaction of hyperchrysophilia and the second phase of axonorrhea, or discharge of the nervous material into the muscle fibers, are intermingled. The discharged nervous material may be either close to or at a considerable distance from the terminal axons of the motor end-plates. The arrangement of the secretion in close proximity to the end-plates is unipolar, bipolar, and multipolar. The structure of the secreted material is likewise variable, namely: (1) as discrete granules, (2) as vacuoles, (3) as short or elongated fusiform masses, and (4) as short or long arrow-like projections. The internal structure of the masses of Kühne's granules projected from the motor end-plates into the muscle fiber may be either densely opaque or cross-striated. The cross striations, when present, may or may not agree in periodicity with the related cross striations in the muscle fiber. These projected axonic masses of Kühne's granules are seen both within and without the muscle fiber. This is more clearly revealed in the studies of the cross-sections of the muscle fibers. There is a torrential projection of axonic material that, for a short period, floods the contents of some muscle fibers. This excessive discharge of the nervous secretion from the motor end-plates is detected by the superabundance of gold-staining masses that vary in size and configurations. The ephemeral architecture and multiplicity of designs of the discharged nervous secretion in muscle are consistent with the various steps occurring in the dissolution of the secretion and its progressive incorporation into the substance of the myoplasm. There is, therefore, no constant and fixed line of demarcation between the motor nerve ending and cross-striated muscle. This variation in composition of the junction between the secretory nerve ending and muscle has made the search for an absolute line of separation between the two structures vain; there is no such constant partition between nerve and muscle.  $\times 300$ .



## PLATE 40

FIGS. 11 to 13. Sprays of axons of medullated nerve fibers and motor end-plates (Figs. 11 and 12) of the gastrocnemius muscle of the white rat, 30 seconds subsequent to the heat stimulus of a 10-second exposure in water at 90° C. The second phase of the neuromuscular reaction, axonorrhea, is clearly manifested. Elongated streamers of gold-staining axonic material discharged from the motor end-plates into the muscle fibers are clearly evident. Some of these projected streamers of nervous material discharged into the muscle are over 1000  $\mu$  in length. At the other extreme of the variation in size, spheroidal granules, 0.5 to 1  $\mu$  in diameter, are to be found. There is, likewise, considerable variation in the distribution of this nervous secretion in the muscle fibers (Fig. 11). This is due to the irregularity of the nervous discharge as well as to the tenuous and ephemeral nature of the nervous secretion. The form of the nervous discharge (Kg.) is clearly evident: elongated fusiform masses, elongated arrowheads (shaped like an isosceles triangle, Fig. 12), or elongated Indian clubs (Fig. 13). There is, therefore, irregularity of the pattern of the discharged nervous material. The stimulus of heat applied to the skin likewise has a neoformative influence on the design of the motor end-plates in muscle. From some end-plates (Figs. 11 and 12) there is a vigorous outpouring of nervous secretion, impregnated black by gold, which forms elongated streamers in direct continuity with the end-plates. Figures 11 and 12,  $\times 150$ ; Figure 13,  $\times 300$ .



# PLATE 41

FIGS. 14 to 17. Sprays of axons of medullated nerve fibers and motor end-plates (Fig. 14) of the gastrocnemius muscle of the white rat, 30 seconds subsequent to the heat stimulus of a 10-second exposure in water at 90° C. The second phase of the neuromuscular reaction to heat applied to the skin, axonorrhea, is clearly manifested. The nervous secretion discharged from the motor end-plates is distributed both close to and at a considerable distance from the end-plates. Some of the secreted material is in the form of fine granules to the right of the lower motor end-plate (Kg., Fig. 14). The elongated streamer of nervous secretion to the left of the lower motor end-plate (Kg., Fig. 14) is 450  $\mu$  in length. In some places it is cross-striated and in others densely opaque. Some motor end-plates are fortuitously caught in the phase of pouring out a shower of nervous material into the muscle fiber. This is manifested (Figs. 15 to 17) in the various magnifications of the same motor end-plate and muscle fiber. Extending to the right of the discharging motor end-plate there is a flood of secreted nervous material, evident as dark, projected masses, into the myoplasm of the muscle fiber. There is morphologic evidence of a great flow of nervous material into the muscle by the streamlining of the very fine muscle striations. Figures 14 and 15,  $\times 150$ ; Figure 16,  $\times 300$ ; Figure 17,  $\times 750$ .

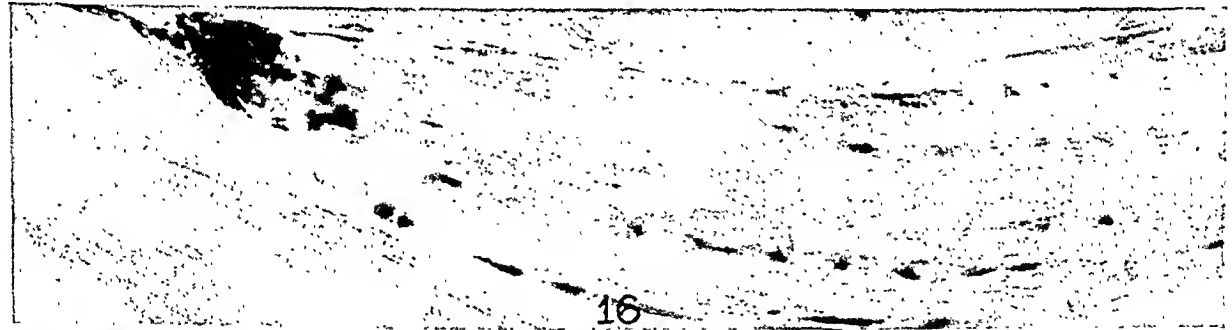
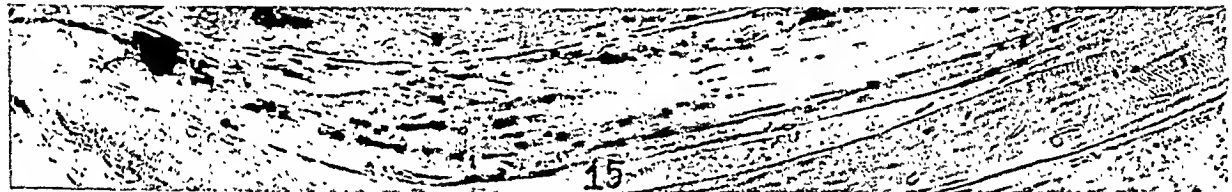
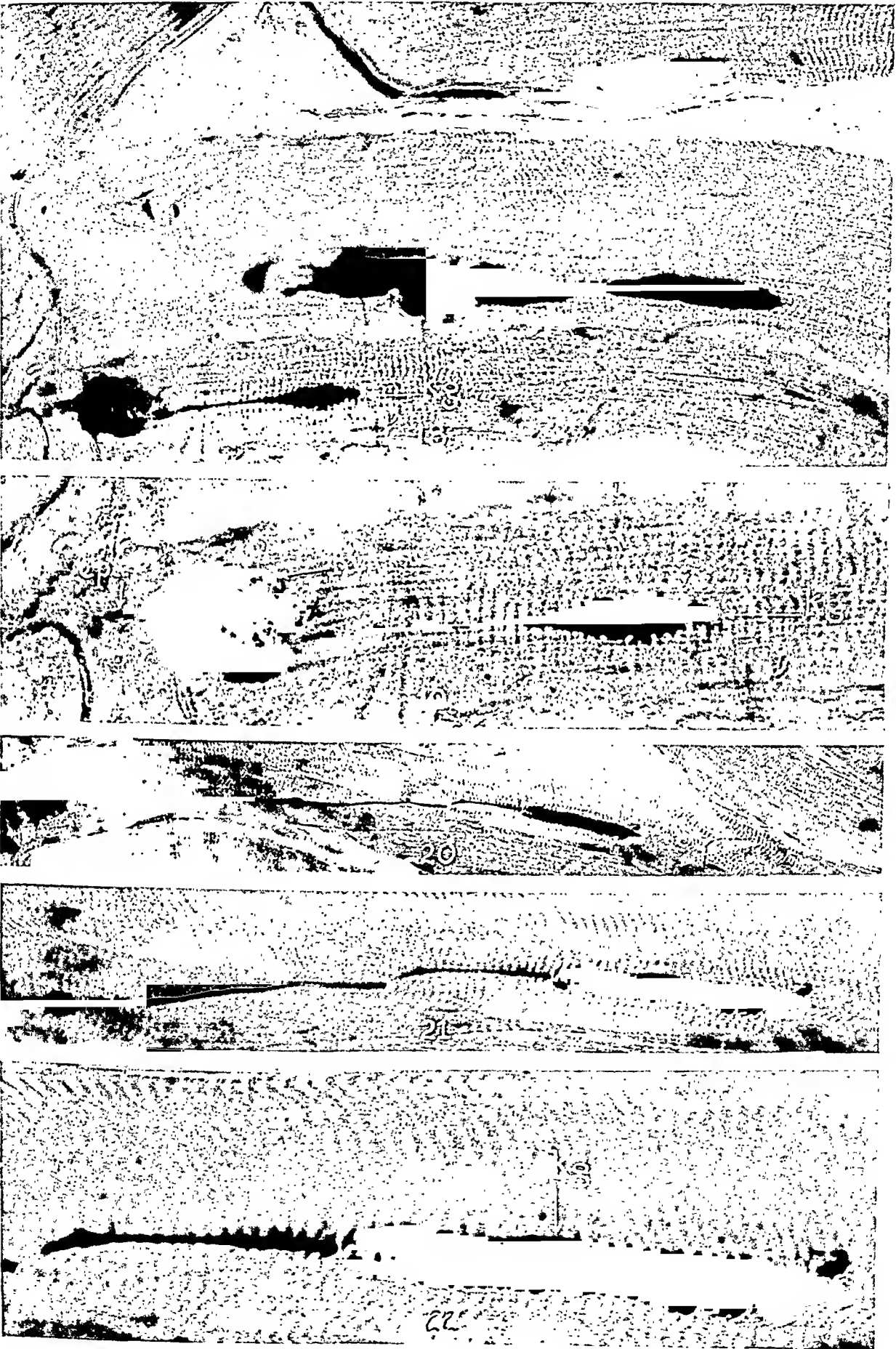


PLATE 42

FIGS. 18 to 22. Ultraterminal projections of axonic masses from the motor end-plates (Figs. 18 to 22) of the gastrocnemius muscle of the white rat, 30 seconds subsequent to the heat stimulus of a 10-second exposure in water at 90° C. The second phase (axonorrhea) of the neuromuscular reaction to heat applied to the skin is manifested by many variations in structure. The term "ultraterminal" is applied to an unmyelinated projection which arises from the axonic material of the motor end-plate and which terminates in the same or in a neighboring fiber. Terminations of both types may be observed. The termination may be globular, fusiform (Figs. 18 and 19), or penniform (Figs. 20 to 22). There is an elongated axonic strand that connects the hypolemmal axons of the motor end-plate with the projected terminal mass. There is a definite streamlining of the labile cross striations of the muscle fiber in immediate relation to the terminal swellings (Figs. 20 to 22) of the ultraterminal projections of axonic material into the muscle fiber. These ultraterminal projections are intermediate phases in the discharge of the secretion of nervous material from the motor end-plates into the muscle fiber. At a slightly later stage the attenuated strand connecting the motor end-plate and the discharged mass undergoes solution. The continuity, therefore, of the discharged masses of Kühne's granules with the motor end-plate is an early stage in the manifestation of the secretion of nervous material into the cross-striated muscle fiber. This overflow of projected nervous substance into the muscle fiber is the result of overstimulation and augmented permeability of the terminal axons of motor nerves in muscle. The delay in the dissolution of the excessive nervous discharge may be due to the local increase of lactic acid and other metabolites in the overstimulated muscle. The continuation of this vigorous outpouring of the nervous fluid into muscle results in structural exhaustion and complete disappearance of the motor end-plates. This produces early denervation of muscle at the myoneural junction. Figure 18,  $\times 300$ ; Figure 19,  $\times 750$ ; Figure 20,  $\times 200$ ; Figure 21,  $\times 400$ ; Figure 22,  $\times 750$ .



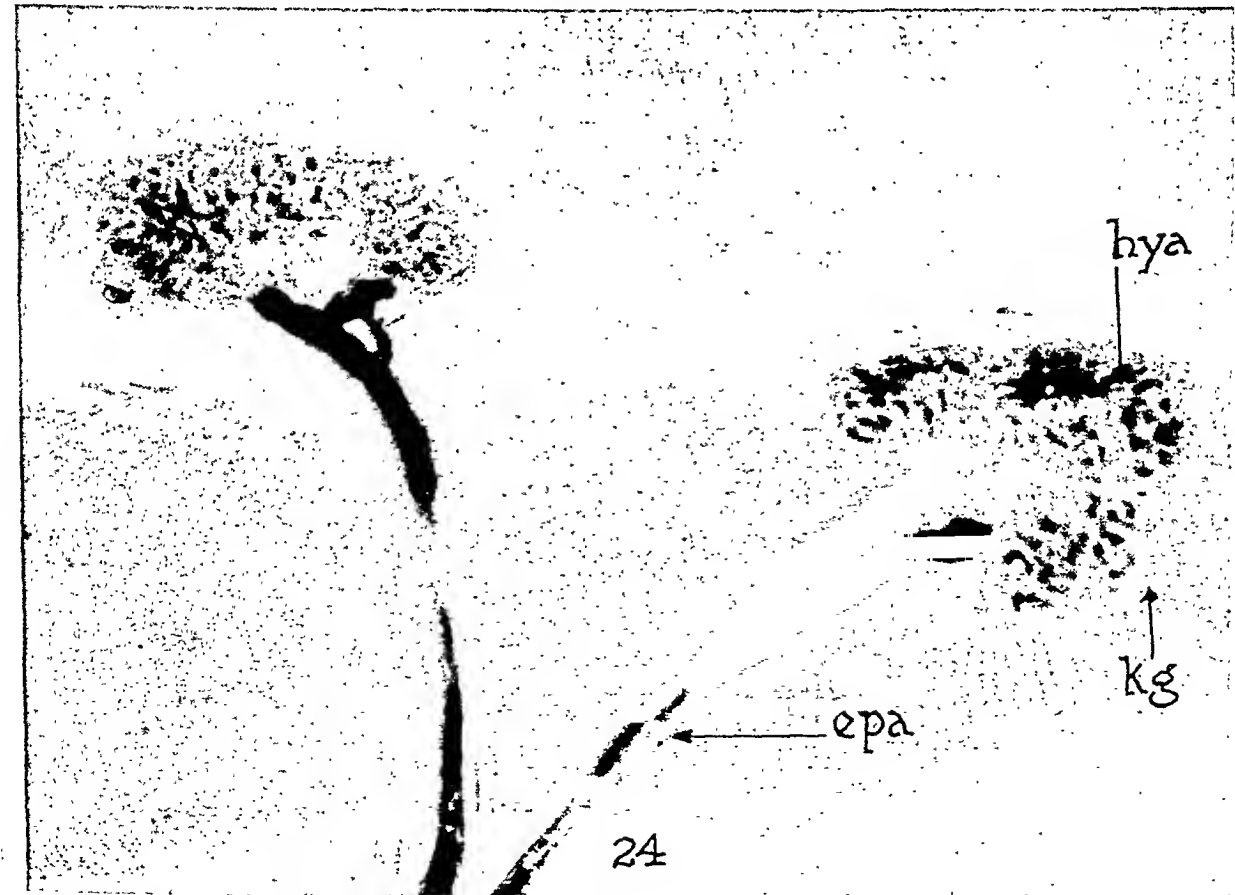
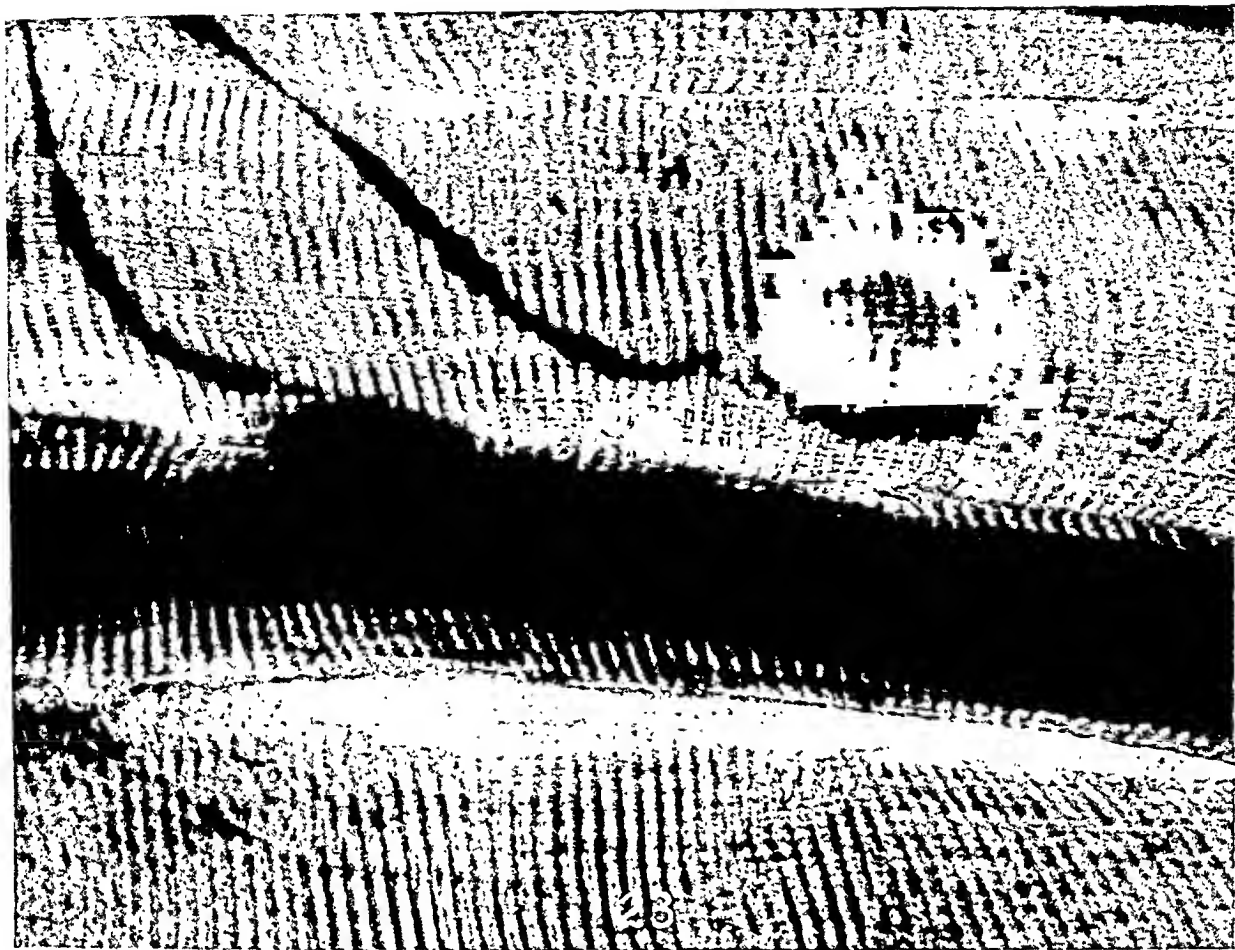
Carey, Massopust, Zeit, and Haushalter

Motor End-Plates in Thermal Shock



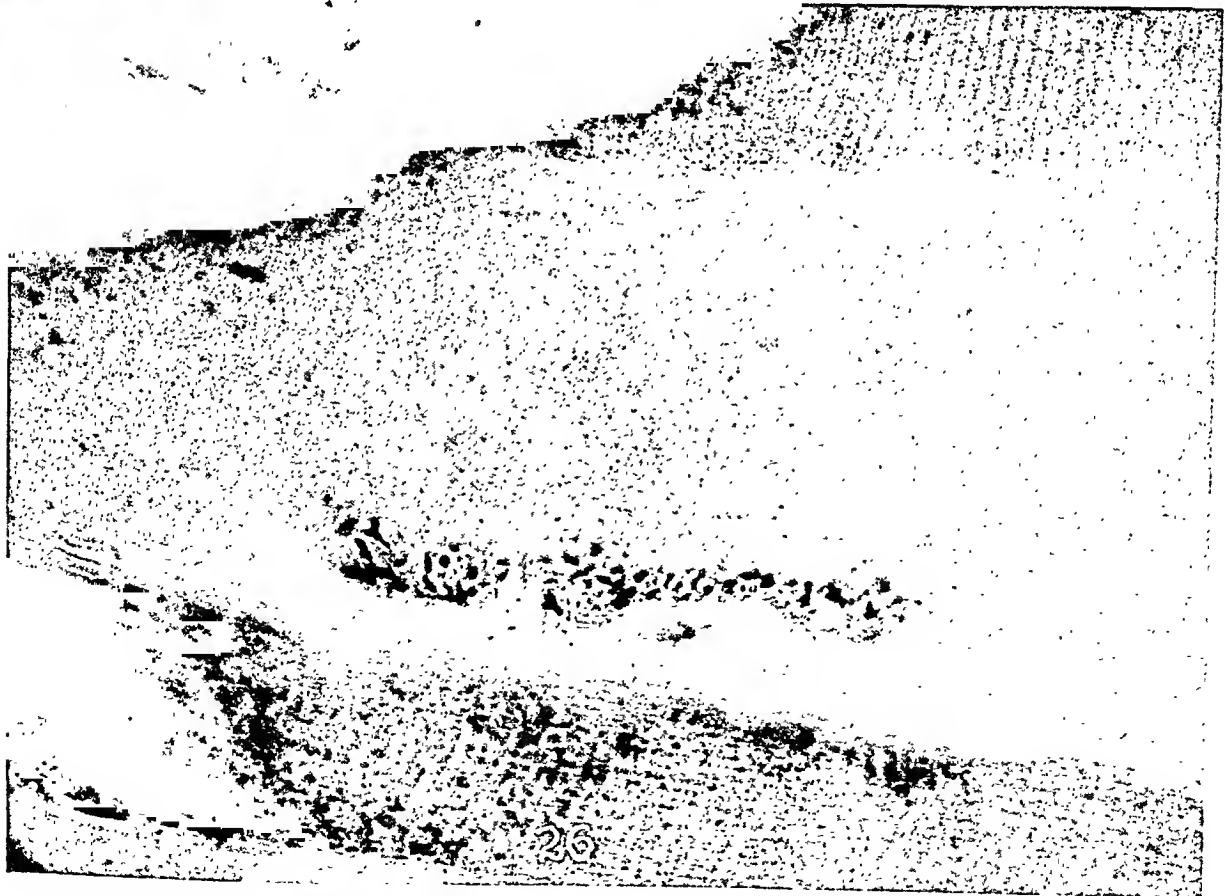
PLATE 43

FIGS. 23 and 24. Sprays of axons of medullated nerve fibers in the relatively normal gastrocnemius muscle of the white rat, excised 15 minutes after the intraperitoneal injection of nembutal. There are variations in the width and types of muscle fibers in the relatively normal muscle. Some of the muscle fibers are narrow (Fig. 23) and densely impregnated with gold. Others are wide and less densely impregnated, giving a light appearance. There are many intermediate gradations in the width of the muscle fibers between the two extremes. The dark cross striations in the narrow muscle fibers are frequently broad, and the light banding narrow. The light, wide muscle fibers (Fig. 24) usually have fine, narrow cross striations. There are variations, however, in the internal structure of the muscle fiber depending on whether the muscle is fixed in the condition of isometric or isotonic contraction. The narrow muscle fibers may show a variation of fine striations, and the wide muscle fibers may show striations of the broad type. These labile cross striations, therefore, are not fixed, static membranes that form permanent partitions in the muscle. They are as variable as the functional activity and the chemical concentration and composition that underlie the activities of the muscle. The motor end-plates in the narrow muscle fibers are retracted and take a dense stain with gold. There are variations in the degree of expansion of the axonic terminals of the motor end-plates. There is a decrease in the quantity of the granules of Kühne around the expanded end-plates. There is a concomitant attenuation of the axonic branches in the expanded motor end-plates. Retracted motor end-plates have been associated with relaxation and expanded end-plates with contraction of the muscle fiber. The motor end-plates in this relatively normal control muscle are of the type designated as "*terminaison en plaque*."  $\times 750$ .



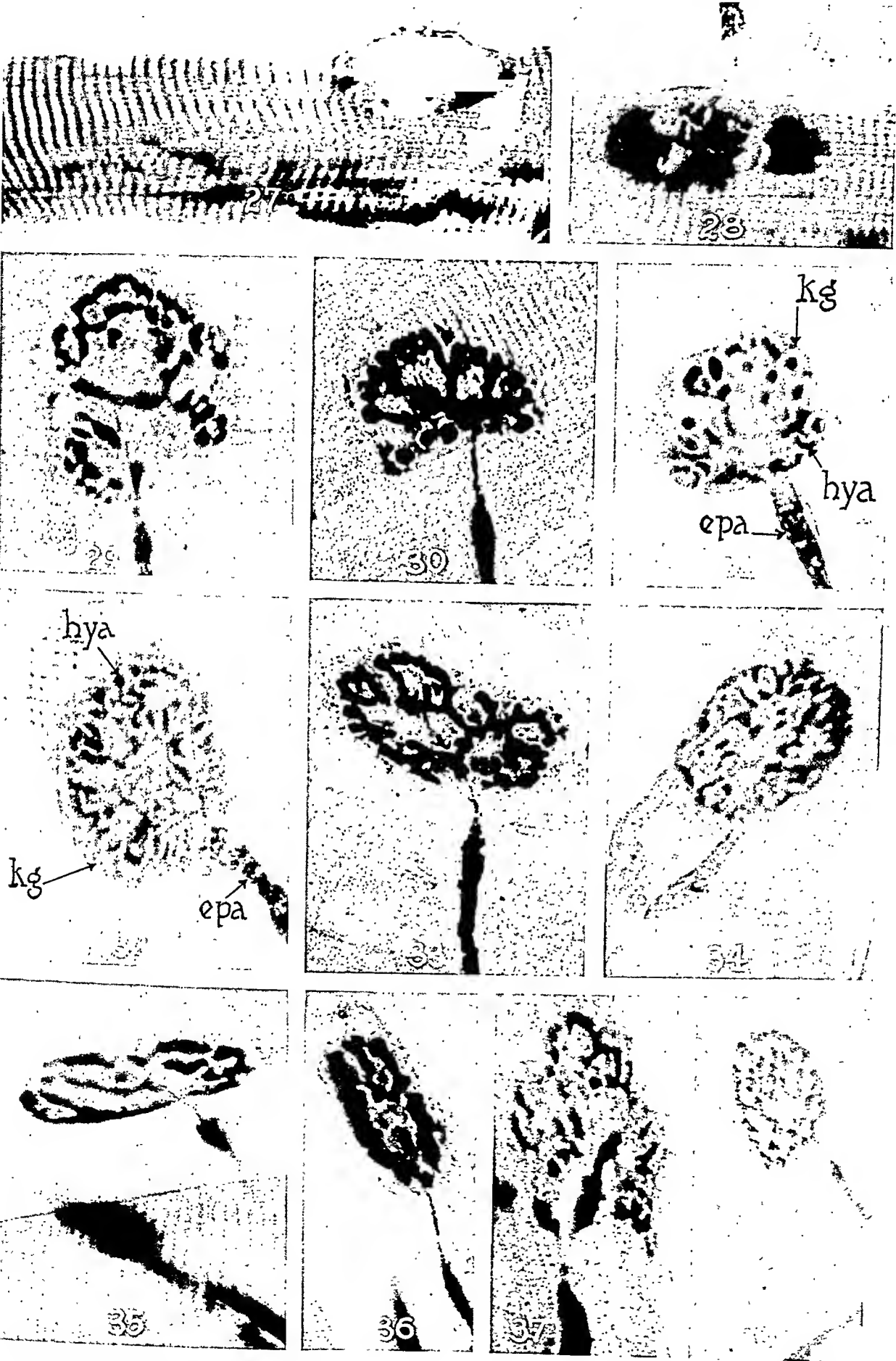
#### PLATE 44

FIGS. 25 and 26. Sprays of axons of medullated nerve fibers in the relatively normal gastrocnemius muscle (Fig. 25) of the white rat, excised 15 minutes after the intraperitoneal injection of nembutal. The motor end-plates in this relatively normal muscle are of the type designated as "*terminaison en plaque*." Some of the end-plates (Fig. 26) in the gastrocnemius muscle, excised 10 seconds subsequent to the heat stimulus at 90° C. of a 10-second immersion of a rat, have the grape-like terminals designated as "*terminaison en grappe*." These grape-like terminals are an exhaustion phase of the ordinary motor end-plates due to overactivity and oversecretion in response to the heat stimulus. They are frequently associated with a muscle fiber that has fine, closely spaced, dark cross striations and with an unmedullated epilemmal axon. The demyelination of the epilemmal axon is, likewise, associated with exhaustion due to overstimulation. The reversible structure of the motor end-plates is due to variations of functional activity. The grape-like motor nerve ending has attenuated and, in many places, discrete swellings of the fine axons, which terminate in little ball-like masses. There is a depletion or complete absence of the surrounding granules of Kühne. The unmyelinated epilemmal axon of this grape-like nerve ending is out of focus when the ending is clearly in focus.  $\times 750$ .



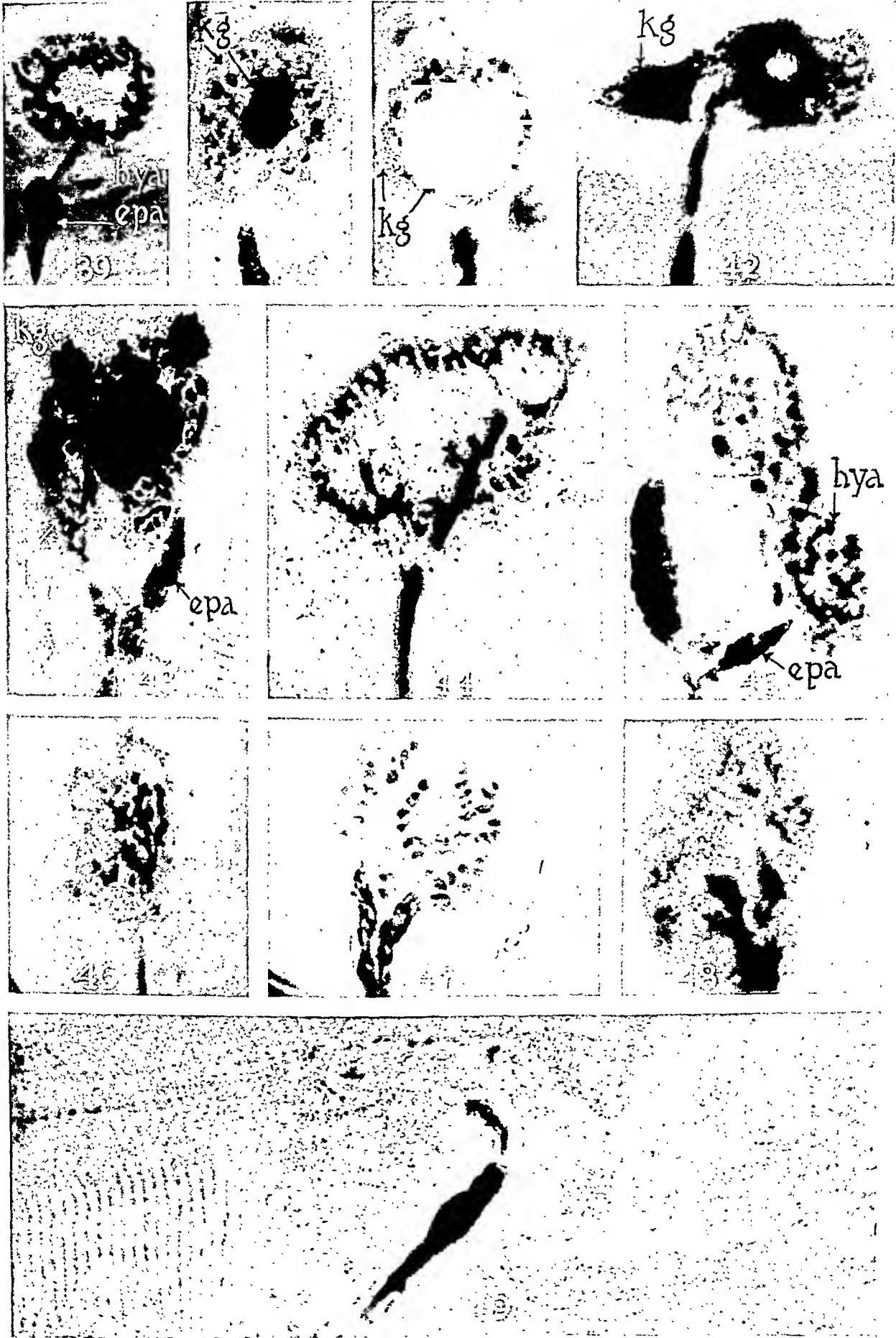
## PLATE 45

FIGS. 27 to 38. The pleomorphism of the normal motor end-plates is evident from these photographs made from the gastrocnemius muscle of the white rat in the relatively normal state, excised 15 minutes after the intraperitoneal injection of nembutal. Many of the retracted nerve endings (Figs. 27 and 28) are surrounded by a dense rim of Kühne's granules and are related to narrow muscle fibers that contain coarse, widely spaced cross striations. There is a great variety of configurations of the expanded motor end-plates. Some have coarse hypolemmal axons (Figs. 29, 30, 33 and 36) related either to fine cross striations or to a complete replacement of the striations by diffuse granulation. Other expanded nerve endings have very narrow hypolemmal axons and a great diminution or complete depletion of the granules of Kühne. The granules of Kühne constitute a fine spray of secretion discharged from the axons in the expanded nerve endings in some locations (Figs. 29, 31, 32, 36). These minute granules of Kühne become quickly incorporated by diffusion and dissolution into the myoplasm. Under normal conditions the neuronc secretion into muscle is exceedingly tenuous and ephemeral. Therefore, it has been hard to detect and interpret some of the clear, oval spaces between the branches of the axons. In the external granular rim they are occupied by nuclei of the granular sole. These nuclei are rendered more clearly visible by counterstaining with hemalum or hematoxylin. This counterstaining, however, frequently smudges the definition of the other parts of the neuromuscular apparatus. In some places there is a clear, halo-like space between the axon and the granules. In others there is a direct continuity between the granules and the axons. It is clearly evident that the architecture of the neuromuscular apparatus under relatively normal conditions is characterized by great variation and irregular patterns. The pleomorphism of these motor end-plates is due to variations of amoeboid motion of retraction and expansion, and material exhaustion due to secretion. The epilemmal axon is irregularly beaded.  $\times 750$ .



## PLATE 46

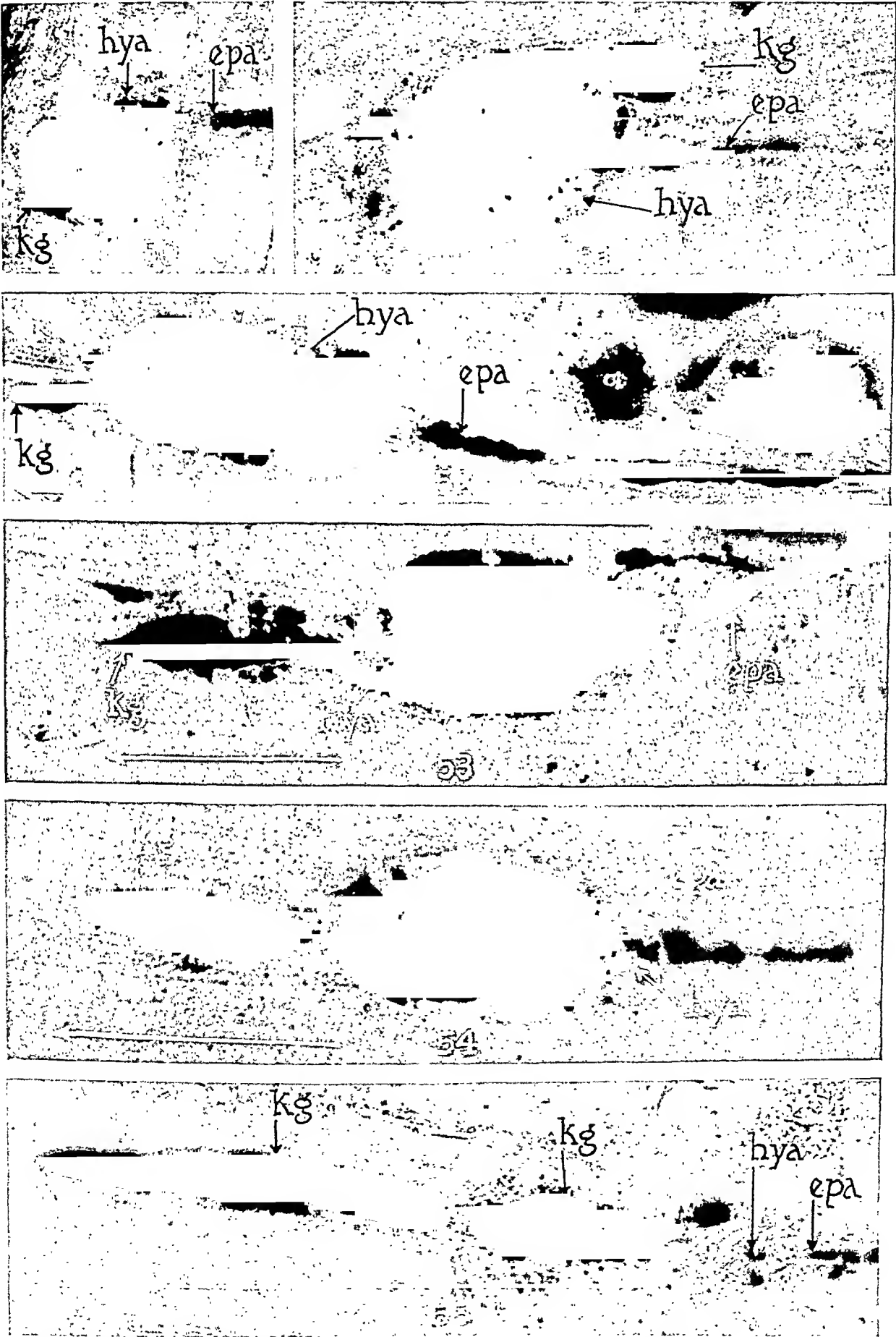
FIGS. 39 to 49. The pleomorphism of the motor end-plates is clearly evident, also, in the gastrocnemius muscle of the white rat, excised 30 seconds subsequent to the heat stimulus of a 10-second exposure in water at 90° C., applied to the skin. The hypolemmal axons may form a complete ring open in the center (Fig. 39), or may contain dense, rounded masses of Kühne's granules that form either a small (Fig. 40) or a large hub (Fig. 41). This central mass of Kühne's granules may form a ring with a thick rim (Fig. 42). There may be irregular distribution of the rounded islands of Kühne's granules (Fig. 43). There may be large rings (Fig. 44) or irregular rings (Fig. 45) formed by the expanded axons of the motor end-plates. The hypolemmal axons of the motor end-plates become gradually more and more attenuated as they become transformed into the granules of Kühne (Figs. 44 to 48). There is fragmentation (Fig. 47) and complete transformation of the hypolemmal axons of the motor end-plates into granules (Figs. 48 and 49). In this explosive transformation (Fig. 49), there is, likewise, a replacement of the periodic cross striations into an irregular mass of granules in close proximity to the end-plate which is undergoing rapid liquefaction. The epilemmal axons have irregular configurations in relation to the descent of the axonic material into the hypolemmal axons. The structural loss of innervation at the myoneural junction is due to the transformation of the terminal axons into granules. The rapid diffusion and incorporation of the granules into the myoplasm results in peripheral nervous exhaustion by depletion of the nervous axonic fluid discharged into muscle.  $\times 750$ .





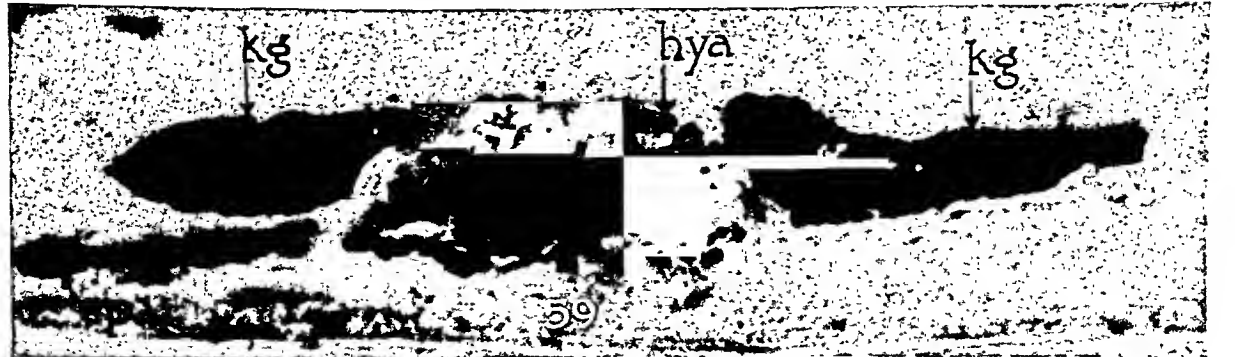
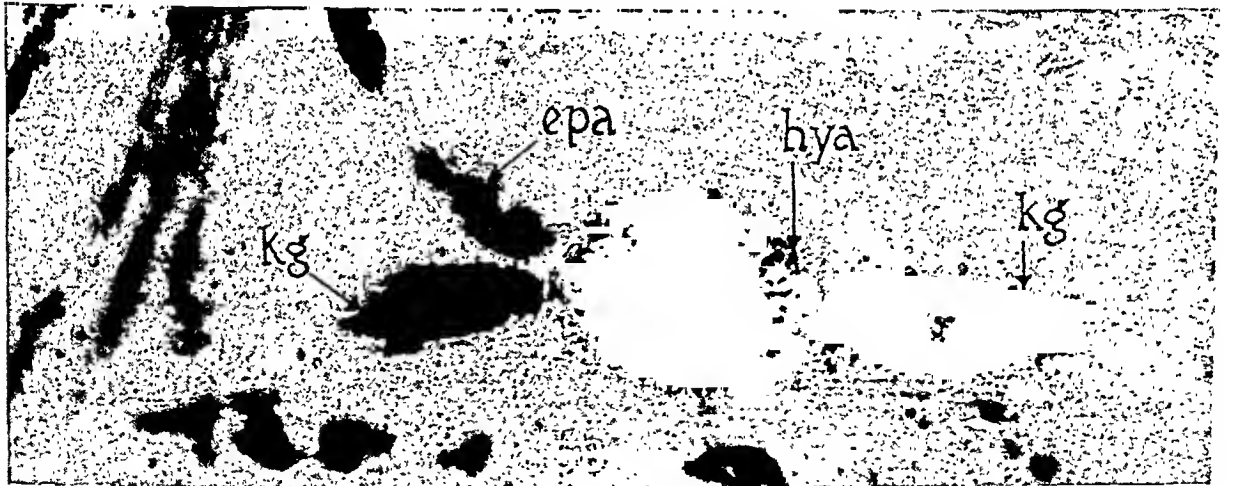
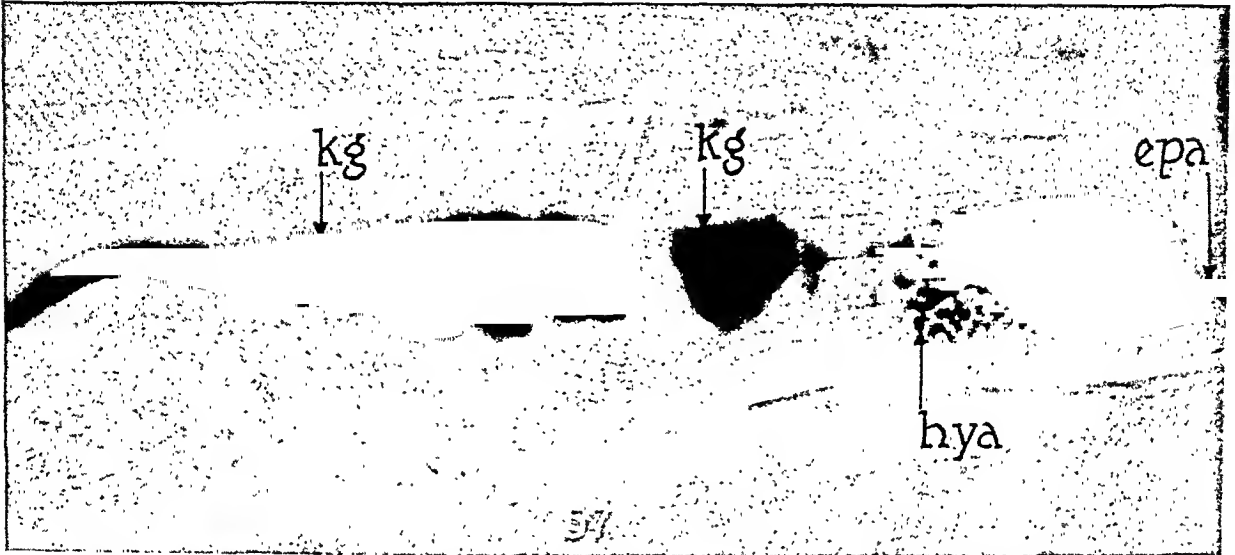
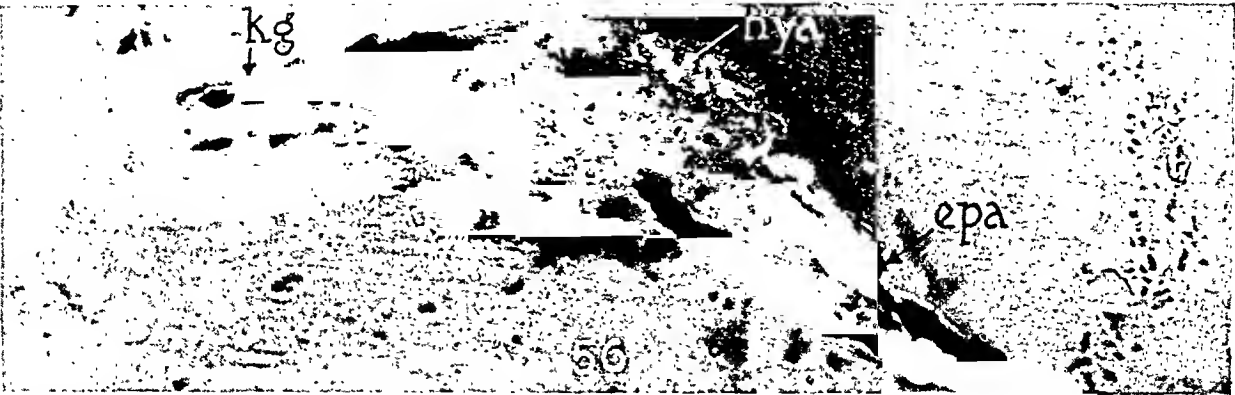
## PLATE 47

FIGS. 50 to 55. The pleomorphism of the motor end-plates is illustrated further from the gastrocnemius muscle of the white rat, excised 30 seconds subsequent to the heat stimulus of a 10-second exposure in water at 90° C., applied to the skin. The hypolemmal axon is in a condition of hyperchrysophilia due to the augmented accumulation of axonic material that has a specific affinity for the impregnating gold. This condition is not due to overstaining. The intense impregnation with gold is due to the fact that there is an increased quantitative accumulation of the axonic material in the end-plate, which material has an affinity for gold. There are various phases in the discharge of this nervous material into the muscle fiber by direct continuity from the motor end-plate. The elongated extensions of Kühne's granules may occur as short projections (Figs. 50, 51, 52), or as unipolar fusiform projections (Figs. 53 and 54), or as a duplex series of fusiform projections (Fig. 55), related to a depleted motor end-plate (Fig. 55). The fine cross striations are granular and closely spaced (Figs. 53 and 54) or they have undergone a complete replacement by a diffuse arrangement of granules (Figs. 50, 51, 52, and 55).  $\times 750$ .



## PLATE 48

FIGS. 56 to 59. The pleomorphism of the motor end-plates is illustrated from the gastrocnemius muscle of the white rat, excised 30 seconds subsequent to the heat stimulus of a 10-second exposure in water at 90° C., applied to the skin. The hypolemmal axon is in a condition of hyperchrysophilia due to the augmented accumulation of axonic material that has a specific affinity for the impregnating gold. This condition is not due to overstaining. There is a unipolar disposition (Figs. 56 and 57) of the discharged masses of Kühne's granules to the left of the motor end-plates. There is a bipolar disposition of the discharged granules of Kühne (Figs. 58 and 59) to the left and to the right of the motor end-plates. The motor end-plate forms an open ring (Fig. 56) in some places, and in others the axonic ring of the end-plate encloses a dense island of discharged granules of Kühne (Figs. 57 to 59). In some places the cross striations are exceedingly fine and closely spaced (Fig. 57). In such places there is a streamlining effect produced on the pattern of the cross striations. This is evidence that these striations are easily displaced by the material from the nerve ending that becomes incorporated into the myoplasm of the muscle fiber. In other locations (Figs. 56, 58, and 59), the fine cross striations are undergoing replacement by a diffuse arrangement of granules. The alteration of the irregular configurations of both the motor end-plate and the cross-striated muscle occurs instantaneously and with explosive violence. By overstimulation of the neuromuscular apparatus, reflexly produced by heat applied to the skin, a flood of liquid nervous material with a variety of patterns is discharged into the muscle.  $\times 750$ .



#### PLATE 49

FIGS. 60 to 62. The pleomorphism of the motor end-plates is clearly evident in the gastrocnemius muscle of the white rat, excised 30 seconds subsequent to the heat stimulus of a 10-second exposure in water at 90° C., applied to the skin. There is great variation in the width of the epilemmal axons (Fig. 60) in relation to the motor end-plates. This variation is consistent with the periodic descent of axonic material into the motor end-plates. The discharge of great masses of this material from the nerve ending into the muscle may be multipolar in arrangement (Figs. 61 and 62). The hypolemmal axons of the motor end-plates decrease in size in direct proportion to the transformation and discharge of the granules of Kühne. It is clearly evident that the heat stimulus applied to the skin has an immediate neoformative influence on the structure of the motor end-plate. The variations of design of the neuromuscular apparatus are related directly to variations of physiologic and pathologic activities. The relative disposition of the parts of the motor end-plates is not static and fixed, but is dynamic and highly variable in its architecture.  $\times 750$ .

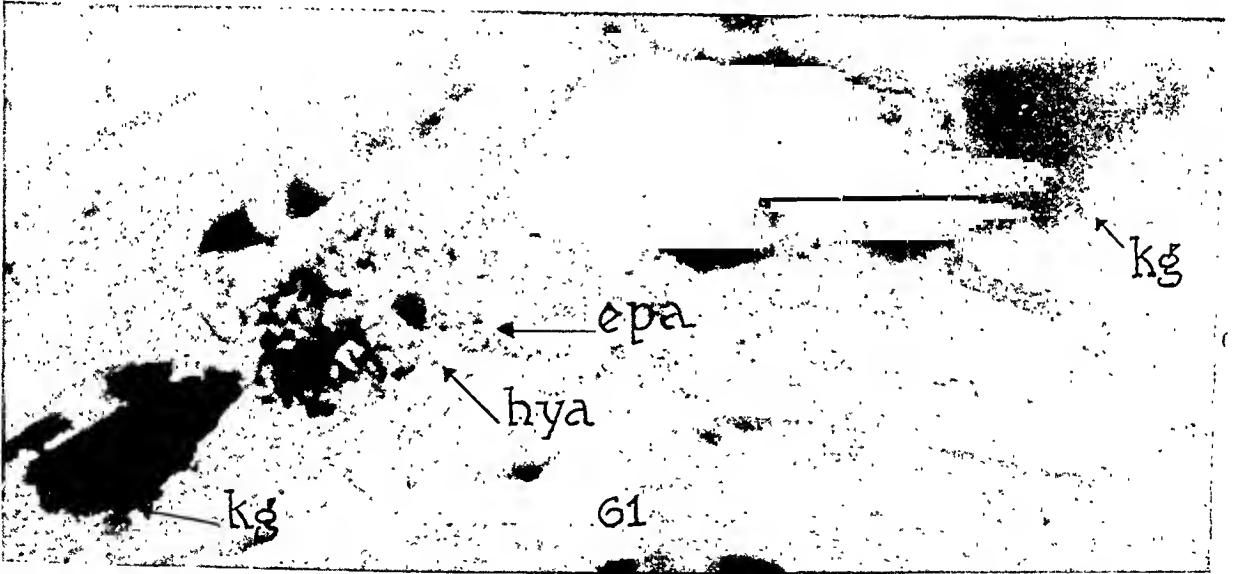
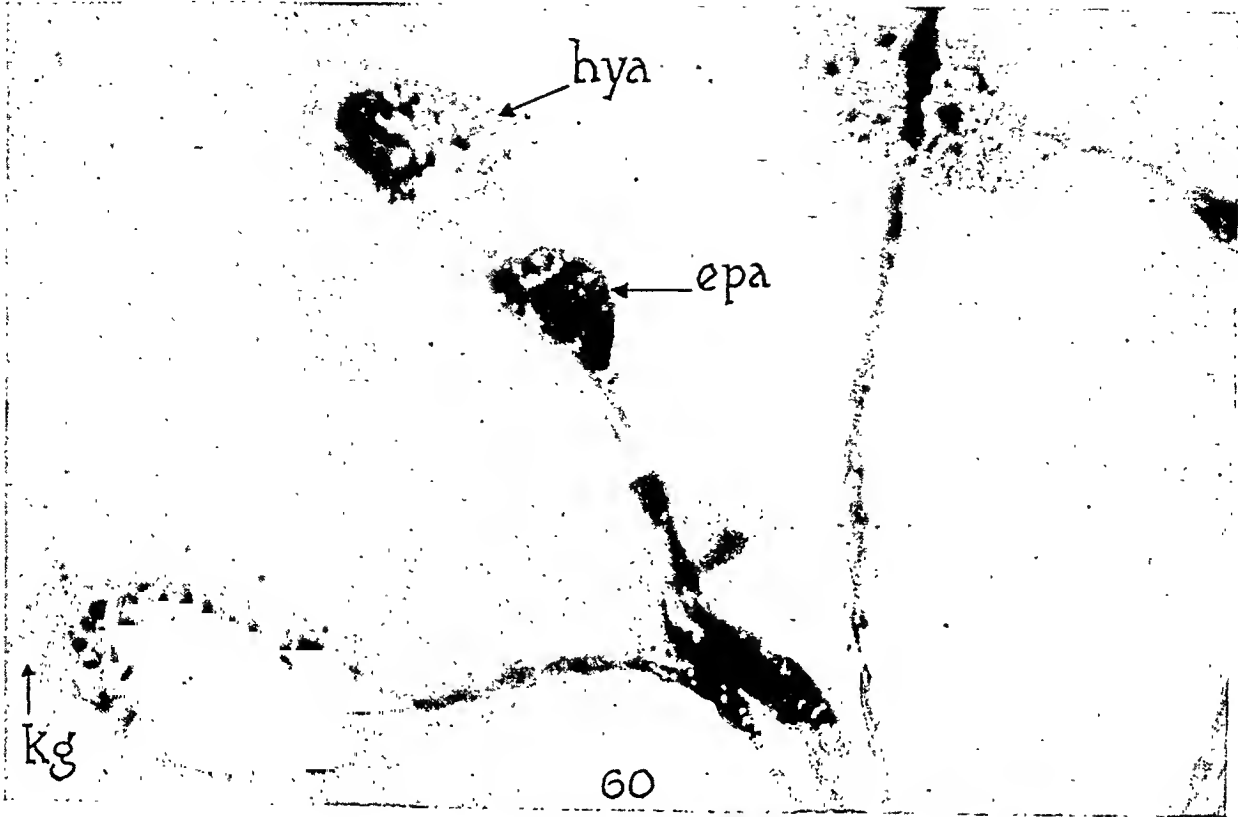
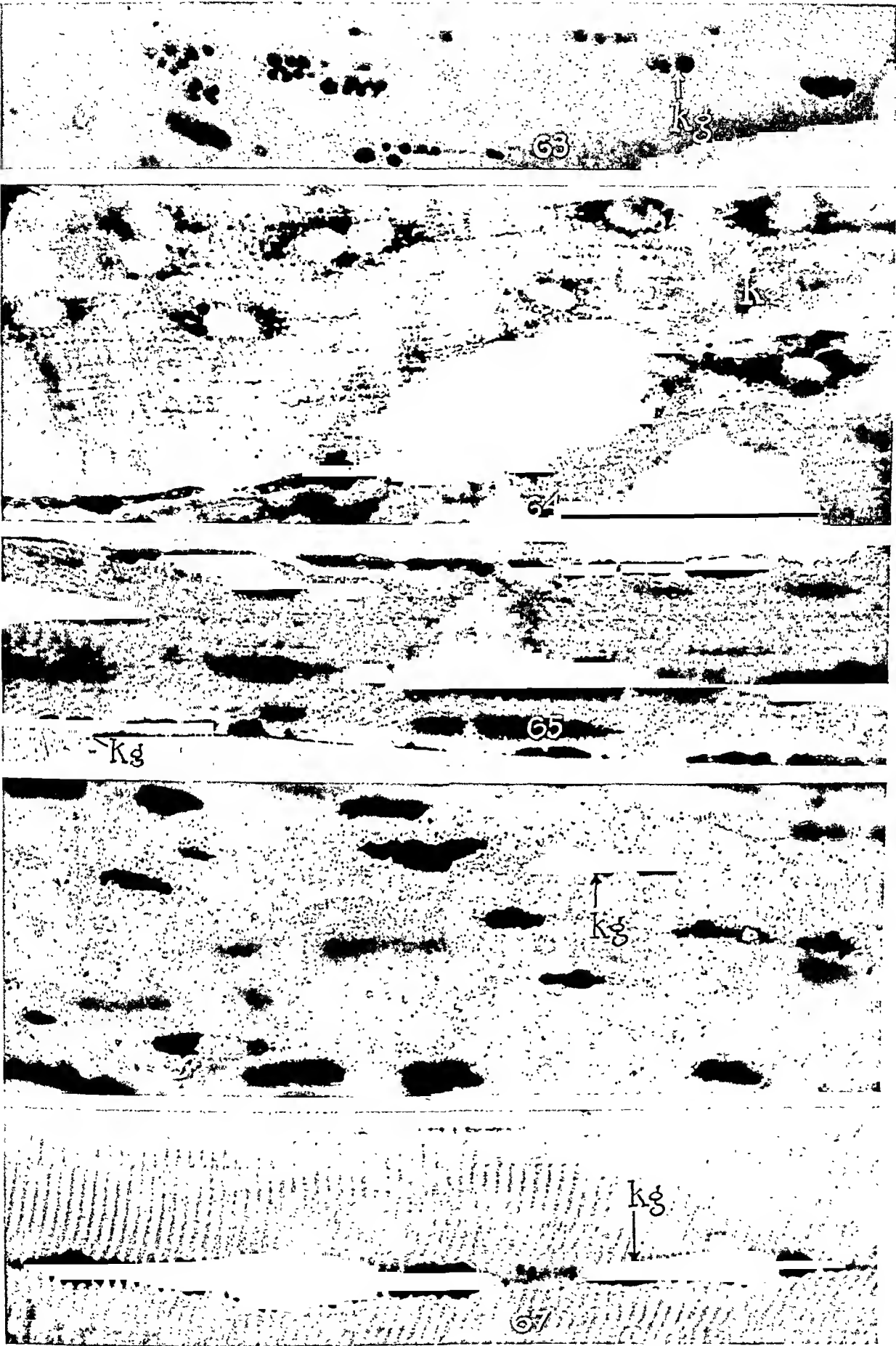


PLATE 50

FIGS. 63 to 67. The pleomorphism of the discharged granules of Kühne into the muscle fiber is clearly evident. These discharged granules are arranged in a variety of patterns, namely: discrete granules arranged in clumps or in series (Fig. 63); discrete or duplex vacuoles (Fig. 64); and elongated streamers or irregular masses (Figs. 65 to 67). It is clear that some of these masses lie within the muscle fiber and underneath the sarcolemma (Fig. 65). In other places they are found between the muscle fibers. In many locations the discharged secretion of the motor end-plates is dense and opaquely impregnated with gold. In other places these masses of Kühne's granules are cross-striated and their pattern of striation may or may not agree with that of the muscle fiber. These masses of nervous secretion discharged into the muscle undergo progressive dissolution and incorporation into the colloidal substance of the myoplasm of the muscle fiber. It is clearly evident, therefore, that there is no fixed boundary or line of demarcation at the junction of nerve and muscle. The substantial secretion from the motor nerve-ending becomes incorporated into the explosive mixture that forms muscle. The neuronic secretion into muscle may be compared roughly to the mercury fulminate that causes the explosion of black powder.  $\times 750$ .





### PLATE 51

FIGS. 68 to 70. Sprays of epilemmal axons depleted of motor end-plates, 3 hours after scalding the skin of the rat in water at  $75^{\circ}$  C. for 5 seconds. There is both a hypochrysophilia and an achrysophilia corresponding to the progressive dissolution of the motor end-plates. The terminals of the epilemmal axons form enlarged ends. The hypolemmal axons have disappeared. In the previous location of the epilemmal axons are clusters of dark, pyknotic, rounded nuclei, 6 to 15 in number. These nuclei of the original granular sole of Kühne appear to be modified nuclei of the muscle fiber. Within the cluster of nuclei of the depleted motor end-plates there is granulation of some of the cross striations of the muscle fiber. These morphologic changes are comparable to those produced by the injection of lactic acid locally into the zone of innervation of the muscle. Gold chloride and hemalum stain.  $\times 750$ .

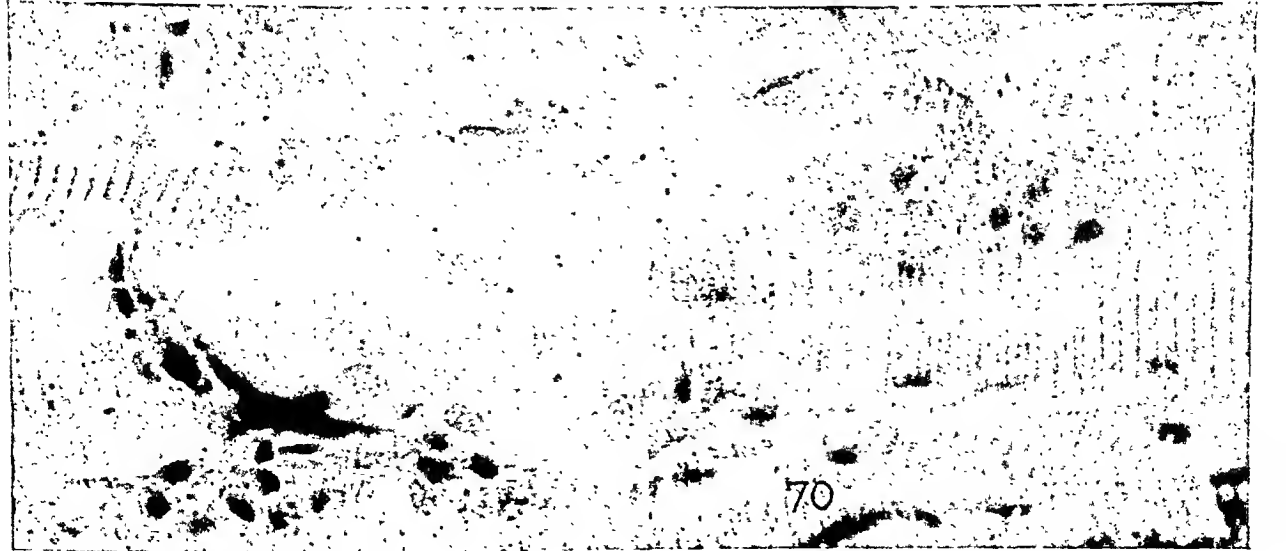
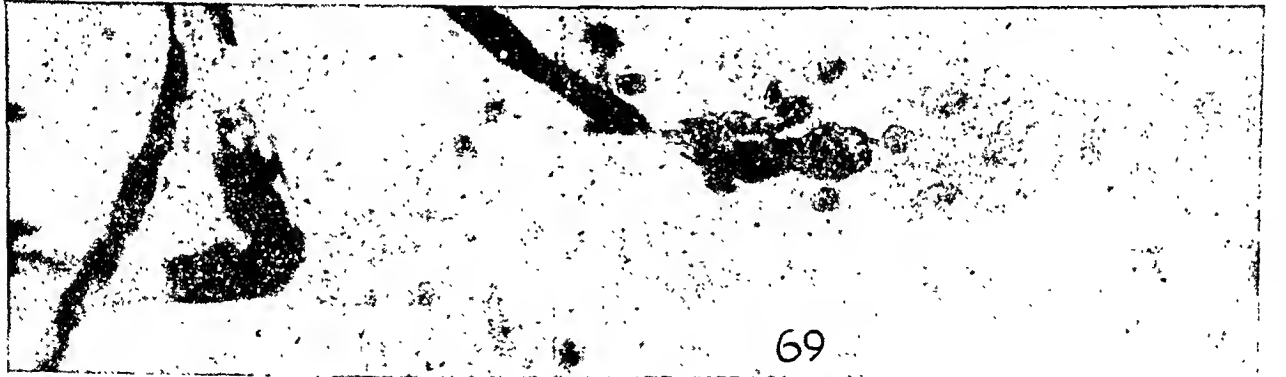
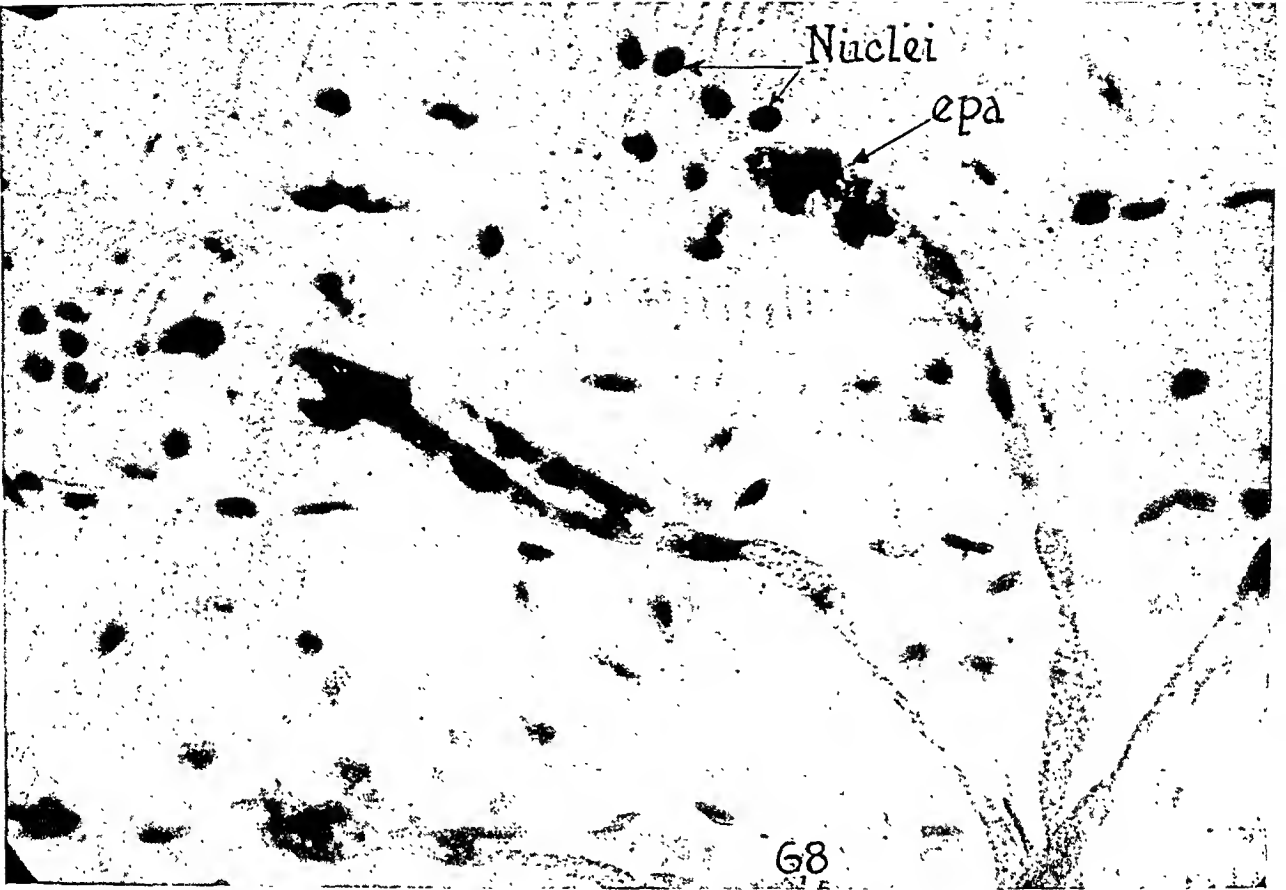
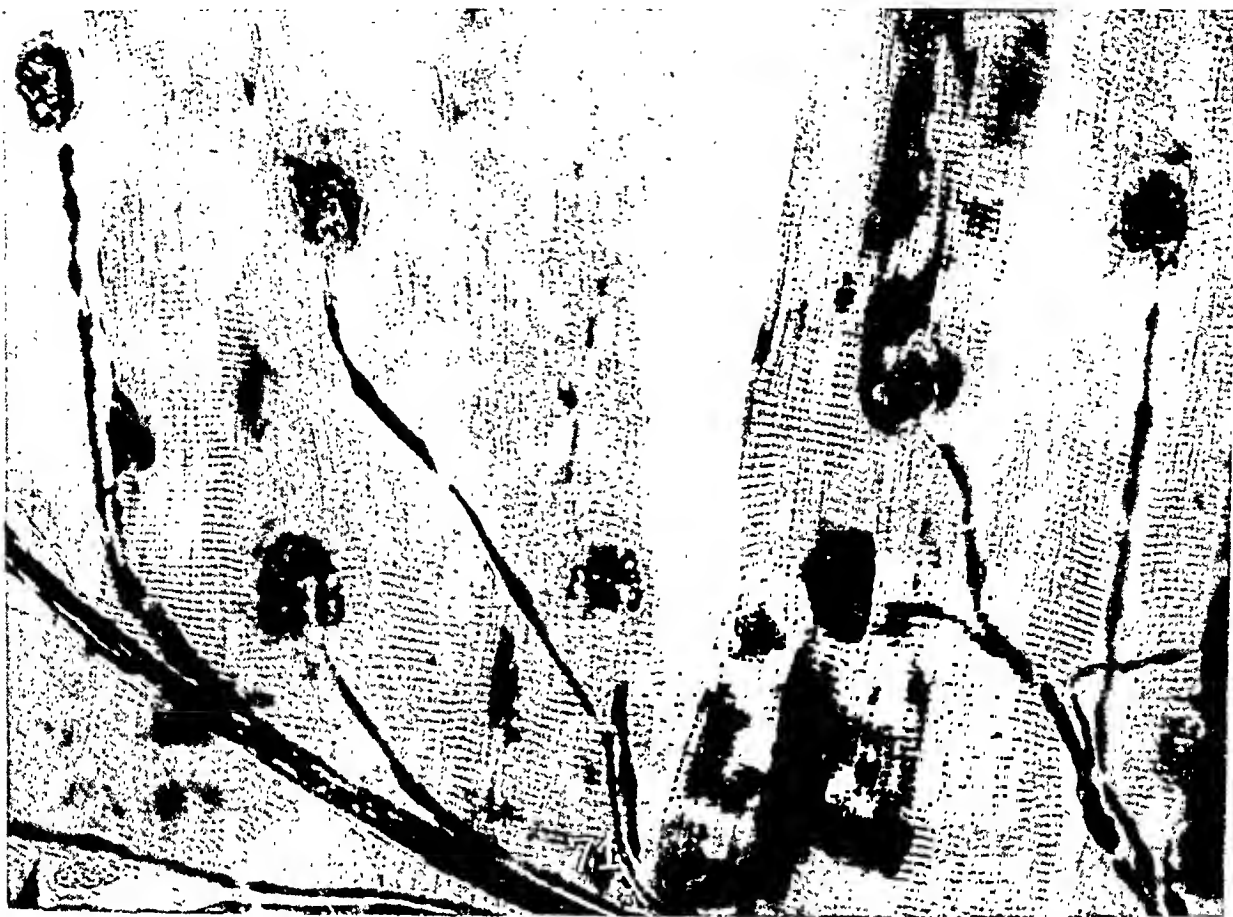


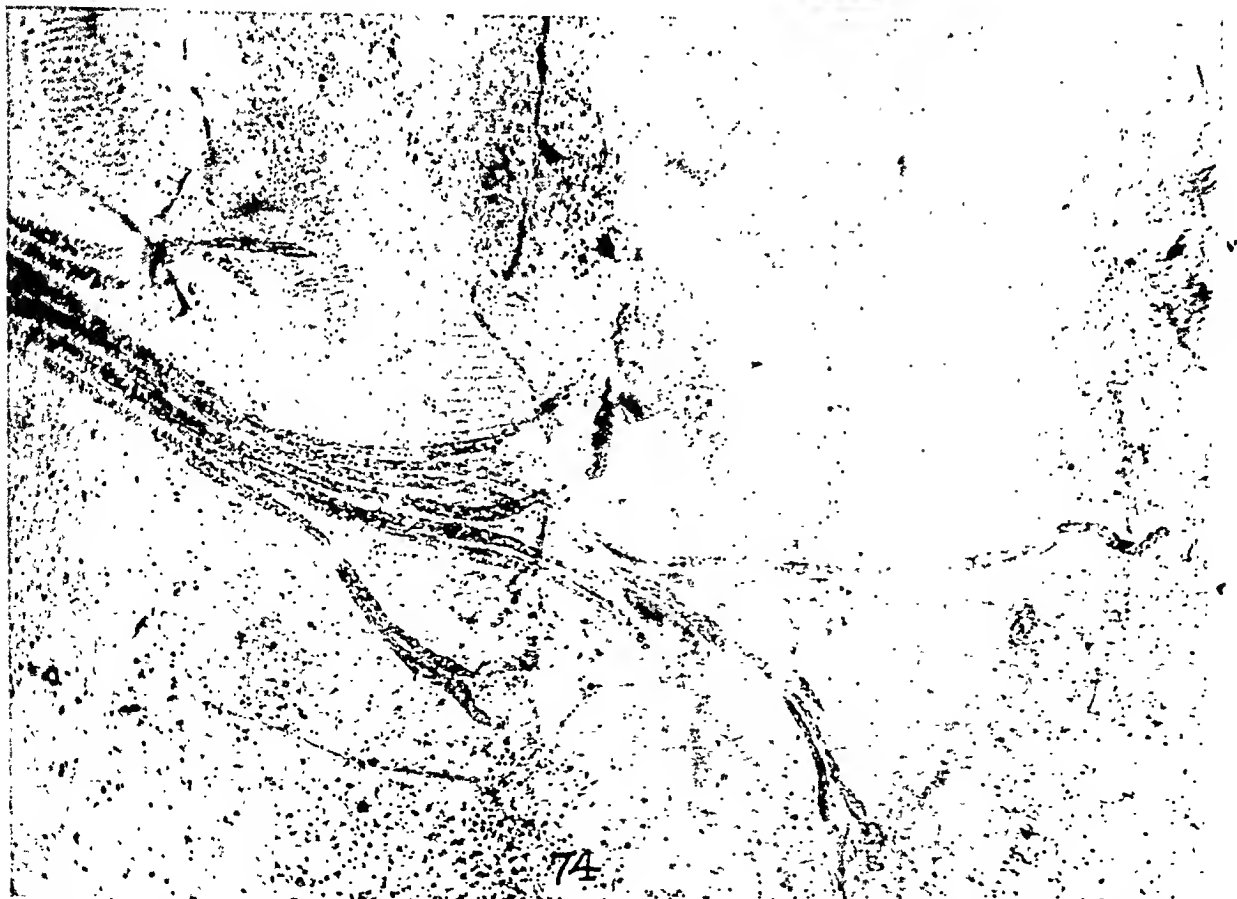
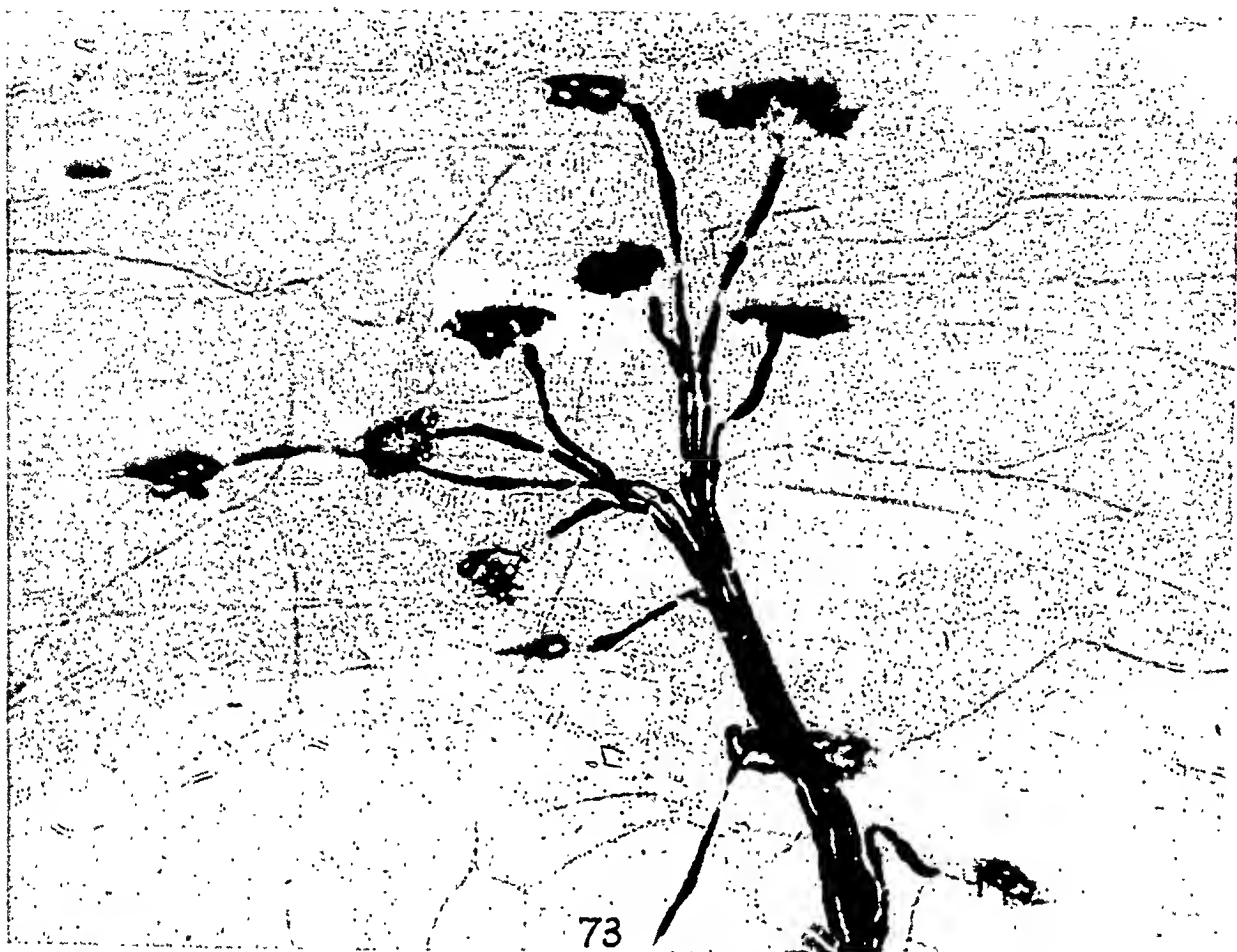
PLATE 52

FIGS. 71 and 72. Sprays of axons of medullated nerve fibers of motor end-plates of the gastrocnemius muscle of the white rat, in the relatively normal state, excised 15 minutes after the intraperitoneal injection of nembutal. The variations in the structure of the relatively normal motor end-plates and muscle fibers (Fig. 71) are obvious. The retracted nerve endings are surrounded by a dense rim of Kühne's granules, blackened by impregnation with gold. In one location there is an irregular, halo-like space between the axons of the end-plate and the rim of Kühne's granules. The majority of the end-plates are expanded and have diminution or complete absence of Kühne's granules. The epilemmal axon, although irregularly beaded, is intensely impregnated with gold. The progressive dissolution of the motor end-plates (Fig. 72) is found 2 hours after scalding the skin for 10 seconds in water at 75° C. There is a progressive hypochrysophilia that terminates in complete achrysophilia of the motor end-plates during their progressive dissolution. The internal structure of the muscle fiber is composed of fine cross striations undergoing various stages of granulation.  $\times 300$ .



### PLATE 53

FIGS. 73 and 74. Sprays of axons of medullated nerve fibers of motor end-plates of the gastrocnemius muscle of the white rat, in the relatively normal state, excised 15 minutes after the intraperitoneal injection of nembutal. There is a variation in the structure of the relatively normal motor end-plates (Fig. 73). The majority, however, are in a state of expansion, and the related muscle fibers are composed of exceedingly fine, closely spaced cross striations. The clearly defined epilemmal axons have an intense affinity for the impregnating gold. This normal morphologic state (Fig. 73) is in striking contrast to the motor end-plates and epilemmal axons from the gastrocnemius muscle (Fig. 74) excised 2 hours after the entire body of the rat (except the head and neck) had been immersed for 10 seconds in water at 75° C. There is a complete disappearance of the motor end-plates, the previous location of which is characterized by achrysophilia or absence of impregnation with gold. There is an irregular cluster of granules that occupies the location of the liquefied motor end-plates. There is a hypochrysophilia of the epilemmal axons. The depletion of these epilemmal axons of their gold-staining material extends in a centripetal direction away from the granular sites of the previous location of the motor end-plates. Exposure of the skin surface to water at a scalding temperature results in a thermal shock, part of the lesions of which are reflected in the neuromuscular apparatus. There is a denervation of the muscle fibers due to the structural disappearance of the motor end-plates and epilemmal axons. The study of the sequence of changes from the normal state to the stage of complete structural exhaustion reveals the secretory mechanism of the neuromuscular apparatus.  $\times 300$ .





# THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XXII

MARCH, 1946

NUMBER 2

## GYNECOMASTIA \*

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The literature covering enlargement of the male breast has been reviewed splendidly up to 1930 by Kriss<sup>1</sup> and up to 1933 by Menville.<sup>2</sup> The condition was known to Aristotle. Galen first used the term "gynecomastia" (woman, breast) to indicate an increase in the amount of fat underlying the breast.<sup>3</sup> Gynecomastia, whether corresponding to Galen's conception or not, was described by Paulus Aegineta, Fabricius ab Aquapendente, Paracelsus, Haly Abbas and others of mediaeval times.

### *Definitions*

Galen's definition applies only to what is now called pseudogynecomastia. Various authors have referred to gynecomastia in terms of the breast of females. For example, Weber<sup>4</sup> spoke of it as mammary feminism, and Cheatle and Cutler<sup>5</sup> defined it as "an affection of the male breast in which the gland tends to assume the size, shape and sometimes the functions of the female breast." Similar views as to the nature of the lesion have been expressed by Bronstein,<sup>6</sup> by Maciel Crespo,<sup>7</sup> by Ilabaca Leon,<sup>8</sup> and by Word and Reed.<sup>9</sup>

Fifty years ago, however, Stieda,<sup>10</sup> basing his definition partly on etymology, said that the term "gynecomastia" is justified only by external form and volume, that microscopically the tissue neither corresponds to nor closely simulates the functional breast of the female.

Other writers have referred to gynecomastia as hypertrophy of the breast; these include Deaver and McFarland,<sup>11</sup> Maliniac,<sup>12</sup> Dunn,<sup>13</sup> and others. It is listed in the Quarterly Cumulative Index under the heading "Hypertrophy of the Breast." Ewing<sup>14</sup> used the term "gynecomastism" to include hypertrophy of the male breast with or without changes in the testicle. Williams,<sup>15</sup> in one of the early discussions, said that gynecomastia differs from hypertrophy in the female in that there is no tendency to indefinite increase in size; growth ceases when it attains the dimensions of the normal breast of the female. Others, such

\* Received for publication, May 10, 1945.



as Webster,<sup>10</sup> avoided controversy by referring to the condition simply as enlargement of the male breast.

Menville<sup>2</sup> said that much confusion has resulted from a failure to define clearly the limits of gynecomastia. It may be added that if the term "hypertrophy" merely means increase in size, it may be properly applied, but if it carries in addition the connotation of increased functional capacity, it may not be. The belief that the process duplicates the breast of the female is incorrect, as is the assumption that it represents feminization, if that term is used in its full meaning. Many men with gynecomastia have all other morphologic and functional evidence of masculinity (Figs. 1 and 2). Gynecomastia differs from adenofibroma, chronic cystic mastitis, mazoplasia, and other mammary diseases of the female. Further confusion is caused by failure to distinguish pseudogynecomastia, a deposition of fat in or under the breast, from the hyperplasia of true gynecomastia. Introduction of the term "anisogynecomastia" (Del Castillo, de la Balze, and Reforzo Membrives<sup>17</sup>) to indicate inequality of bilateral enlargement has done nothing to lessen the confusion. One of the purposes of this communication is to arrive at a definition which will separate gynecomastia from inflammatory diseases and neoplasia of the breast of the male.

### *Pathologic Anatomy*

The altered mammary gland is described as a button-like or plate-like mass, or a nodule, under the nipple, rarely attached to the nipple, but not adherent to other structures. The mass may become fairly large, attaining a weight of as much as 600 gm., and appear as a more or less globular body. Several authors have stated that it may reach the size of the breast of a virgin—whatever that may be. The mass is firm, with smooth outline, and may be tender or painful, or both. During the process of growth, which goes on for a few months or years, the enlargement is ordinarily gradual, but in a few instances there may be periods of interruption or acceleration. After it reaches a certain size, growth ceases. Secretion occurs in a few cases, appearing either spontaneously or on expression, but this secretion has not been proved to be milk.

In most cases the nipple and areola show no departure from the normal. In a few there may be variable degrees of enlargement and projection of the nipple, widening of the areola, sometimes with increased pigmentation, and prominence of the glands of Montgomery.

The mass is firm, of smooth outline, clearly but not sharply defined; it offers considerable resistance to cutting and in cross section is firm, slightly bulging, moist, pale gray or bluish gray, fairly homogeneous, often translucent, and sometimes slightly lobulated. The mass merges

fairly abruptly with the surrounding adipose tissue, but is not definitely encapsulated. Even when considerably distended, the ducts are not visible in cross section, but in rare instances cysts a centimeter or more in diameter are seen.

The microscopic picture is not described as well as the gross in the literature. Gill<sup>18</sup> said that the mammary tissue resembles that of a girl 12 or 13 years old. Both Goodman<sup>19</sup> and Geschickter<sup>20</sup> compared the lesion to the breast of the female at puberty; zum Busch,<sup>21</sup> to the secreting mammary gland.

Other writers have compared gynecomastia to mammary disease in the female. Sullivan and Munslow<sup>22</sup> regarded the lesion as almost identical with chronic cystic mastitis of the female, more commonly of the adenosis type. Menville<sup>2</sup> could find no clear distinction between gynecomastia and fibro-adenoma of women, an opinion in which Lewin<sup>23</sup> concurred. Webster<sup>16</sup> made the confusing statement that gynecomastia is indistinguishable from fibro-adenoma and from chronic cystic mastitis, two lesions which can readily be differentiated. Gill<sup>18</sup> stated that gynecomastia resembles pericanalicular adenofibroma but without whorls of connective tissue. Lewis and Geschickter<sup>24</sup> were of the opinion that gynecomastia differs from adenoma in the absence of acini and lobules, but Erdheim<sup>3</sup> and also Cheatle and Cutler<sup>5</sup> described acini, and Bonn and Evans<sup>25</sup> spoke of imperfectly formed acini. No clearly identifiable acini were found in the material used in this study.

Multiplication of ducts and epithelial proliferation are well described. Erdheim<sup>3</sup> as well as Cheatle and Cutler<sup>5</sup> indicated that the larger ducts are not involved, but that is not true.

Stieda<sup>10</sup> (1895), Erdheim<sup>3</sup> (1928), and Menville<sup>2</sup> (1933) had stated that the increase in size of the breast is due principally to proliferation of connective tissue stroma; but Cheatle and Cutler<sup>5</sup> attributed it to an increase in volume of the entire tissue of the gland. Moore, Wattenberg, and Rose<sup>26</sup> drew attention to the great increase in connective tissue, intimating that it was partially due to edema. Among the specimens in this survey edema was infrequent, the connective tissue bulky, and the periductal tissue loosely arranged.

The presence of lymphocytes, plasma cells, and large mononuclear cells in the periductal tissue and sometimes in the dense stroma has caused little comment. Ingleby<sup>27</sup> described an infiltration of lymphocytes and an occasional plasma cell around many ducts. Menville<sup>2</sup> reported a lymphocytic infiltration with some plasma cells and eosinophils in loose periductal tissue, where Geschickter<sup>20</sup> also noted a moderate number of wandering cells. In photomicrographs incorporated in other publications cellular infiltration is often observed but not referred to in the text.

## PRESENT STUDY

This study was undertaken at the request of the Director of the Army Institute of Pathology, Army Medical Museum, Col. J. E. Ash, M.C., U.S.A., and his associate, Lt. Col. Balduin Lucké, M.C., A.U.S. It is based on protocols and microscopic sections from 284 cases of gynecomastia, nearly all from soldiers, filed in the Army Medical Museum, from the Zone of the Interior and Theaters of Operation. Since beginning this investigation many new cases have been filed but not added to the material utilized as it already represented a fair sampling of the whole collection.

## GENERAL DATA

Data on various clinical aspects were kindly collected by Mrs. Helen Friedman. The information in the protocols is not complete in many cases, but is extensive enough to indicate the characteristics of gynecomastia.

*Age.* The relation of the lesion to age is suggested by classifying by decades the 280 cases in which the patients' ages were recorded.

Age by decades . . . . .	10-19	20-29	30-39	40-49	50-59	60 and above
Number of cases . . . . .	44	192	23	12	7	2

The youngest patient was 14 years old, the two above 60 were 76 and 77 years of age.

*Race.* Of the 228 patients whose race was noted, 193 were white, 32 Negro, 1 each Puerto Rican, American Indian, and Chinese.

In regard to both age and race, the figures do not represent accurately the distribution in the civilian population, and they cannot be corrected statistically because data are not available.

*Breast Involved.* Of the 274 cases in which the location of the lesion was defined, both breasts were affected in 12, the right alone in 138, and the left alone in 124.

The location of gynecomastia has been tabulated in relation to racial distribution as follows:

Race	Breast Involved		
	Right	Left	Bilateral
White	87	79	10
Negro	15	14	
Unspecified	36	29	2
Puerto Rican		1	
Chinese		1	
American Indian*			
Totals	138	124	12

\* In the case of the American Indian the side involved was not stated.

*Duration of Complaint.* From the protocols it is impossible to state in most instances whether the history began with mammary enlargement, pain, tenderness, or trauma. Two patients claimed to have noticed the lesion only 1 week before operation. The following table gives the duration according to the histories:

Duration	Less than 3 mos.	3 mos. to less than 6 mos.	6 mos. to less than 1 yr.	1 yr. to less than 2 yrs.	2 yrs. and more
Number of cases	90	38	27	19	62

Among soldier patients there is the possibility that the matter of service-connected disability may at times distort the histories. More dependable information may be gathered from histories which date back 2 years or more, and for this group the distribution is as follows:

Duration in years	2	3	4	5	6	7	8	10	11	15	Several	Many	Since puberty
Number of cases	14	9	8	4	5	3	1	1	1	2	11	1	2

From these cases the beginning of the enlargement may be roughly estimated. Among those who gave a history of 2 years' duration was a boy of 14 and one of 17 years. All the others were in their twenties, except one who was 56, and the age of one was not given. Of those in the 3-year group, all were in their twenties except one who was 18, and the age of one was not recorded. All of those with symptoms of from 4 to 11 years' duration were in their twenties. One whose history went back 15 years was 29, and another 31 years old. Two of those with a history of "several years" were 30, all the others were in their twenties, except one whose age was not given. One who claimed to have had the lesion "since puberty" was 22, the other 30. The man who said "many years" was 22! The earliest age of onset claimed was 12 years, but in several others it was 13 and 14. A wide variation in stated age of onset is evident.

Duration in cases of bilateral involvement was recorded in only 6, and in 5 of these covered a period of years. A man, 52 years old, claimed the swelling had been present for only 3 months. The relation of duration of the bilateral condition to age is shown in the following table:

Duration in years	2	3	4	5	6	11
Age of patients	20	Not given	25, 20	20	21	23

The Chinese, 27 years old, had unilateral enlargement which had been present 2 years. The Indian, 21 years old, had had unilateral

involvement for "several years." The Puerto Rican, 50 years old, had had his unilateral lesion for 3 months.

*Rate of Progress.* The account of rate of progress of gynecomastia is confusing in the 65 histories in which it is recorded, probably because of misuse of relative terms. The growth is said to have been "slow" in 23 cases, yet the time for this slow growth varies from 2 and 3 months to 6 years. It is said to have been "very slow" in two instances, in one of which the duration was 2 weeks and the other 2 years. Growth was "gradual" in 20 instances, but the time was given as from 5 weeks to "several" years. "Rapid" was applied to 10 cases, the duration being from 1 month to 6 months. "Moderate" was used for 3 cases of from 6 weeks to 6 months. The growth was "progressive" in 3 cases, with durations of from 1 month to 4 years. Two patients, with disease lasting 1 year and 7 years respectively, had "recurrent swelling." One patient had had the swelling for 11 years with rapid growth in the last 4 months; another had had the lesion for 6 years, with rapid increase in size for 1 month.

In spite of the unreliability of histories and interpretations, it is evident that there was progressive enlargement continuing from a few weeks to several years. In only 4 cases was there noticeable periodic alteration of rate of growth.

*Relation of Trauma to Onset.* A history of injury was given in 28 cases and denied in 1. A "blow" received from 3 weeks to 3 years previously was recorded in 11 of the 28 cases. Injury, without specification of its nature, was reported in 8 cases, with a duration of from 4 months to 8 years. Minor injury had been sustained by 1 patient, 4 months before operation. "Possible injury" appeared on 1 record, 2 years earlier. Two patients told a story of "probable injury," each 3 months earlier, and another with a 3-year history had been "bruised" 3 months before operation. One patient struck a board 2 years before, and another was struck in wrestling 3 years before. One patient said the breast was irritated by a gas mask strap 3 months previous to operation. Another, whose lesion appeared 15 years after an injury, said that he sustained a second injury 2 months before operation.

*Subjective Symptoms.* Pain was a complaint in 50 cases in which duration varied from 2 weeks to several years. Pain was qualified as sharp in 1 case (3 months), slight or mild in 4 cases (1, 2, 2, and 3 months), moderate in 1 (10 months), and persistent in 1 (4 months). Tenderness was a symptom in 24 cases, in which the duration of the lesion was from weeks to several years. It was occasional in 1 case

of long standing and extreme in 1 case of 4 years. Pain and tenderness were associated in 6 cases in which duration of the lesion varied from 3 weeks to 2 years. Soreness was reported in 3 instances, in 1 of which the lesion had been present 6 weeks, in another 6 months, and in the third 7 years. Soreness and tenderness were associated in 1 case of unknown duration. Sharp pain was the complaint of 1 patient, whose lesion had been present only 2 weeks. The enlarged breast of 1 patient, in whom the swelling had appeared 4 years before, was "painful if struck." Progressive tenderness and pain accompanied a lesion with a duration of 1 month, and "dull ache" another of 2 months. Patients who had had swelling for 3 months, 18 months, 2 years, and 7 years said that they had been troubled by pain for 1 week, 3 weeks, 6 months, and 1 month, respectively. Pain had been present in 1 case for 3 months, swelling for only 2. In another, tenderness had been noted for the last 2 weeks although the lesion had appeared 10 months before. In the majority of cases the duration of the subjective symptoms was not indicated.

The psychic factor, although it is not specifically named in the records, is important and will be dealt with in the discussion.

*Weight of Tissue.* Most of the weights were determined after arrival of the specimens at the Army Institute of Pathology, and cannot be consistent or representative, since skin and nipple were included in some and only the gland or portions of it in others. For these reasons control weights of normal breasts are of no value. The nodule appeared to weigh from 26 to 100 gm. (11 cases); however, it was less than 25 gm. in 8, between 126 and 175 gm. in 7, and as much as 325 and 369 gm. in 2 cases. In 1 case of bilateral involvement the weights were 135 and 152 gm., whereas in another they were 369 and 25 gm.

*Size of Specimen* varied so widely that figures are worthless. Most measurements are given in the metric system, but a few are indicated vaguely as the size of pullet's egg or of a walnut.

#### MICROSCOPIC FEATURES

Sections were stained by the usual hemalum and eosin method, after fixation in formalin. Nearly all tissues were embedded in paraffin; only a few were frozen sections. Controls were breasts of young men, presumably normal, secured at autopsy. These were furnished in part by Lt. Col. William B. Wartman, in charge of laboratories at Dibble General Hospital, and in part were from material collected at the

Institute of Pathology, Western Reserve University. The description of the various alterations in the breast will follow the following outline:

- Proliferation
  - Connective tissues
  - Epithelial tissues
    - Increased number of lining layers
    - Sprouts or buds
    - Lacunae
    - Budding of ducts
  - Mixed connective and epithelial tissue
  - Papillae
  - Intracanalicular growth
- Secretion
- Inflammation
  - Periductal
  - Perivascular
  - Diffuse
- Neoplasia
- Resolution
  - Desquamation
  - Ulceration
  - Healing
- Apocrine glands
- Dermatitis

### *Proliferation*

*Connective Tissue.* The enlargement of the mammary gland in gynecomastia is due principally to proliferation of connective tissue. Although adipose tissue is present, in true gynecomastia it does not contribute significantly to the size of the breast. The connective tissue mass shades into the surrounding substance of the region without sharp definition or indication of encapsulation.

In gynecomastia, it is possible to distinguish a stroma, usually of dense collagenous tissue, and periductal connective tissue, the former occasionally arranged loosely because of edema. The stroma differs from that of normal controls only in amount. Fibroblasts were encountered in the stroma in several cases, always in association with some degree of what appeared to be inflammation. Thus, although fibroblastic multiplication must be responsible for augmenting the connective tissue, mitotic figures are rarely seen, and then may possibly be a sequel of inflammation.

Periductal connective tissue is often loosely arranged, but not because of edema since there is no precipitate in the spaces between fibrils. It is a structural peculiarity without definable cause, which is not constant in gynecomastia, nor even uniform in all parts of the same section. Indeed, it may occur on one side of a duct and not on the other. Fibroblasts; often with amphophilic or slightly basophilic cytoplasm, how-

ever, are always present in this loose tissue. Here, too, are mononuclear cells (lymphocytes, plasma cells, and large mononuclear cells). Thus, although the tissue may be loose because of active proliferation, an inflammatory component cannot be excluded. The presence of mucoid degeneration in this region will be taken up later. Mitotic figures were not found in the connective tissues in this series.

The presumably normal controls lacked such a clear distinction between loose periductal tissue and dense stroma, although suggestive pictures were encountered.

*Epithelial.* In addition to proliferation of the connective tissue, the ducts are increased in number (Fig. 4) but this varies from specimen to specimen and in different parts of the same section. Ducts may be considerably elongated and with varying degrees of distention and branching (Fig. 3). The ducts in the sections were not actually counted but the cases were divided by rough approximation as containing "many," "moderately numerous," or "few" ducts. In the first group there were 78 cases, in the second, 152, and in the third, 47. Even in most of the cases in the last group, the number of ducts appeared to be slightly greater than in the normal controls. Therefore, in at least 250 cases (88 per cent) there was an appreciable increase in the number of ducts.

Mitotic figures, observed in 37 of the 284 specimens, without exception had normal configuration. They occurred in basal, intermediate, and luminal layers of the epithelium (Figs. 5, 6, and 7). In 2 cases of unilateral gynecomastia, the number of mitotic figures appeared to be in excess of that observed in 2 cases associated with choriocarcinoma of the testis, although it was no greater than after administration of stilbestrol in 1 case. Mitotic figures were not found in any of the controls. No sign of nuclear budding was seen. In accord with most modern opinion, amitotic cell division has not been proved. The multiplication of epithelial cells in gynecomastia is the result of mitotic division.

Epithelial proliferation led to an increase in the number of layers in the ducts until there were often five or six. This process was not uniform in all parts of the same duct and the border of the lumen was often irregular in outline. In addition, delicate masses of epithelium, like papillae but without connective tissue stalks, often projected into the lumen (Fig. 8). In 66 cases these epithelial sprouts were not observed. In 58 of these the number of layers in the ducts did not exceed three; in the remaining 8 there were four or five layers. Initiation of sprout formation was probably not due to distention since the two processes were not regularly associated. The principal factor appeared to be the multiplication of the lining cells, which were usually



cuboidal whether or not there was sprout formation. Slight elongation of these cells was observed, but in only few instances were they cylindrical (Fig. 9).

Frequently the epithelial sprouts fused to form tiny locules in the lumina of the ducts (Figs. 10 and 11), but no material of any kind was found in the locules. Occasionally the proliferated cells filled the entire lumen (Fig. 12). In the gynecomastia of choriocarcinoma and in that which followed administration of stilbestrol, no papillae or locules were observed, nor were ducts entirely filled with cells. The multiplication in these cases led to the formation of five or six layers, but this was seen also in some of the unilateral cases. In the normal controls there were two or three lining layers, but no papillae or locules. Multiplication beyond three layers of cells, epithelial sprouts, and occluded ducts were not observed in the controls, which leads to the conclusion that sprouts, locules, and ducts filled with epithelial cells are abnormal.

In only a few sections was there budding of the ducts, either in the form of narrow prolongations, usually branched, with rounded ends, or of small spherical buds projecting laterally from the walls of the ducts or of their branched prolongations. Small spherical buds occurred in small numbers in the control sections. Nevertheless, in the enlarged male breast the buds, when present, were more numerous than normal. That these small buds may be acini cannot be denied, but since they do not form the grouped acini characteristic of the lobule of the female breast and because they contain multiple layers of epithelium they probably are not acini (Figs. 13, 14, and 15).

*Mixed.* True intraductal papillae, with cores of vascularized connective tissue, were observed in three instances (Figs. 16, 17, and 18). In none of them was there pleomorphism of epithelial cells or invasion. In one case the pathologist in the field suspected carcinoma, because a duct was filled with cells, most of which were desquamated. The duct affected was dilated and the impression was that the papillary projections were secondary rather than primary. Periductal inflammation was present here as well as around other ducts. In no specimen were more than a few ducts involved, in contrast to the condition in multiple papillomas of the female. Therefore, the papillary structure is regarded as the result of the various features of gynecomastia rather than as being neoplastic.

More or less knob-like masses of connective tissue projected into the ducts in nearly all specimens, deforming the outlines of the ducts (Figs. 19 and 20). In only two specimens was this alteration absent and in these gynecomastia was obviously slight. The ducts of the male breast are often somewhat tortuous; therefore, angular indentations are fairly

common in the sections, as illustrated in Figure 21, and the normal controls may show this configuration. They differ from the more or less blunt intracanalicular growths which may produce an outline resembling that of intracanalicular adenofibroma of the breasts of females (Fig. 22). In none of the specimens of gynecomastia did the deformity of ducts equal that commonly seen in the female, nor did the intracanalicular projections ever show the characteristic whorled pattern. Furthermore, as a rule, cellular infiltration is infrequent and sparse in the intracanalicular adenofibroma; in gynecomastia it is common. Thus, the intracanalicular growth in the male cannot be characterized as a manifestation of adenofibroma.

### *Secretion*

Acidophilic hyaline material was observed within the ducts in 19 cases, acidophilic granular material in 39, and acidophilic material, both hyaline and granular, in 15. Basophilic material, found in 12 cases, was always granular. Granular acidophilic and basophilic material was observed in 3 cases; a combination of acidophilic and basophilic material, both hyaline and granular, was seen in 3 cases (Fig. 23). It is highly probable that these materials represent secretion of a sort, rich in protein in acidophilic, and rich in mucin in basophilic masses. Secretion of this kind was found in 91 of the 284 cases and may be presumed to be fairly common in gynecomastia. Hyaline acidophilic masses, small and not numerous, were found in one presumably normal control. Thus secretion of this sort is not strictly normal, at least quantitatively. Mucin-producing epithelial cells were seldom seen (Fig. 25), and in only one instance was there material which morphologically resembled colostrum (Fig. 24). It was in a duct near the nipple and was made up of a slightly basophilic, granular material containing a few cells, each with a pyknotic nucleus and poorly staining, slightly basophilic, finely granular cytoplasm. No vacuoles indicative of fat dissolved in preparation were identified. Thus it is probable that the material is a mucinous secretion containing desquamated degenerate epithelial cells. The evidence available is against the assumption that it is colostrum.

Distention of ducts was noted in 63 cases with accompanying cystic dilatation in 5 cases. For 1 of these (no. 69458) there was only one block. This contained a cyst 9 mm. in diameter, with four or five lining layers of cuboidal cells, which in places showed parakeratosis and keratosis toward the lumen. Many squamous cells were in the cavity. There was no surrounding inflammation. In another case (no. 75271), with moderate epithelial hyperplasia and moderate inflammation, two sections were from the wall of a cyst, at least 10 mm. in diam-

eter. The lining was of large cuboidal cells with deeply acidophilic cytoplasm, such as are seen in abnormal involution (chronic cystic mastitis) of the female.

Definite elongation of distended ducts was seen in 2 cases. No special cause for obstruction could be found in any of the cases of distention, cyst formation, or elongation. No accumulation of secretions was seen, but in all (except the case in which only the cyst was present) there was epithelial proliferation together with subacute and chronic inflammation of the interstitium.

Squamous metaplasia was observed in the cyst noted above (no. 75271) and also in 2 other cases in ducts not notably distended (Fig. 26). Metaplasia is likewise seen in the diseased gland of the female.

In an occasional duct the cells had undergone transformation to large cuboidal or cylindrical types with notably acidophilic cytoplasm and relatively small, fairly dense, centrally located nuclei (Fig. 27). This is similar to the picture in abnormal involution and other mammary lesions in the female. It differs from the normal apocrine gland in degree of stratification, the central situation of nuclei in the cells, the absence of secretory globules and of rounded cell border toward the lumen.

Red blood corpuscles were found in the ducts in a few cases. Whether this was the result of natural bleeding into the ducts, of trauma of operation, or of spreading during preparation of the tissue cannot be determined. In most cases it was probably due to operation, but in a few instances conglutination of the red cells in the ducts of tissues, which otherwise appear to be well fixed, suggested natural hemorrhage (Fig. 28). A brown discharge from the nipple has been reported by some authors, but was not a feature in any case of this series.

Lymphocytes and other mononuclear cells were occasionally present in the ducts (Fig. 29), probably due to inflammation.

The ducts of the nipple were not often included in the sections, but in the few that were there was some multiplication of lining cells, although not to the degree found in the mammary ducts. Sprouts, locules, or papillae were not observed. Basement membrane was often conspicuous, as is normal, but the periductal tissue was not loosely arranged in this region even when the infiltration of mononuclear cells was conspicuous (Fig. 21).

### *Inflammation*

Inflammation in various degrees was constant in this series of cases. For the most part it was periductal, but not infrequently it involved the denser stroma and in a few instances it was perivascular. More deeply

situated inflammation always accompanied periductal inflammation, but the converse was not true.

Near the ducts of the control specimens there were often a few lymphocytes, as well as an occasional fibroblast or capillary. In cases of gynecomastia the periductal tissue was usually loosely arranged and infiltrated with lymphocytes, plasma cells, which were usually numerous, and those large mononuclear cells often called histiocytes, which varied in numbers and proportions (Figs. 30 and 31). Polymorphonuclear leukocytes were observed in periductal tissues in 36 cases, but never independently of mononuclear cells. They appeared in the more remote stroma in only 4 cases in which they accompanied periductal infiltration. Infiltration of eosinophils was found in 11 cases, and in all but one of these there were also polymorphonuclear neutrophils. The eosinophils were both polymorphonuclear and mononuclear, the former predominating. There was in no case such massive or widespread infiltration of cells as to justify a diagnosis of so-called plasma cell or eosinophilic mastitis.

The periductal mononuclear infiltration often extended to some degree into the more solid stroma, but it was absent in 133 cases. In many instances there were only lymphocytes in the interstitium, but in others there were also plasma cells, large mononuclear cells, and, in 4, polymorphonuclear leukocytes. In some cases the infiltration was perivascular (Fig. 32). When cellular infiltrates occurred in the stroma they were also pronounced in periductal situations.

As a part of the inflammatory reaction, fibroblasts were observed in periductal tissues in 241 cases (85 per cent). This fibroblastic proliferation extended into surrounding stroma in 181, but only occasionally formed a conspicuous part of the picture (Fig. 33).

Capillaries were noted in the periductal tissue in 65 cases (Fig. 34). They often appeared to be newly formed, and with the fibroblasts constituted granulation tissue (Fig. 35). Interfibrillar mucoid material was observed in loose perivascular tissues in 30 cases, but the basic stain of the sections was so often inadequate that this figure is probably low. This material, when present, was highly characteristic (Fig. 56) and certainly occurred more frequently than in comparable lesions in the female.

Exudate was almost always confined to the extraductal part of the breast. In a small number of cases exudation of lymphocytes or of polymorphonuclear leukocytes occurred into the lumina of ducts. In 2 cases the continuity of exudate around and within the ducts caused interruption of the lining epithelial layers (Figs. 36 and 37). Since

exudate was always periductal and only occasionally intraductal, it is probable that extension is into, rather than out of, the ducts.

Although periductal inflammation was associated with ducts of the nipple, exudate was not found within them. In general the involvement of these ducts differed from that of other ducts in the mammary gland. The epithelial proliferation, usually present, was comparable in degree with that in the gland, but there was no indentation by connective tissue growth, only moderate surrounding infiltration by mononuclear cells, no polymorphonuclear leukocytes, and no exudate within the ducts. Although periductal tissues were often conspicuously loose in the mammary gland, a corresponding change had not occurred in the nipple of the same breast.

### *Neoplasia*

True neoplasia was not observed. The intraductal papillary growths have already been described but they could not be regarded as neoplastic. In rare cases, elongated cells filled ducts (Fig. 40), as in intraductal carcinoma in the female, but in no case did this occur in more than a few ducts and in none of these was there the slightest sign of invasion. The section of a lesion of about 10 months' duration (no. 89457), from the right breast of a white man, 27 years old, had in its center a mass of proliferated ducts about 6 mm. in diameter (Fig. 38), which was circumscribed but not encapsulated. The ductules were numerous, varied as to size, were lined with four or five layers of cuboidal cells with occasional mitotic figures, and were surrounded by infiltrated mononuclear cells; capillaries were numerous around the ductules (Fig. 39). There is no justification for considering it an adenoma since it differed from the other lesions of gynecomastia only in its focal character. In this whole series, there was nothing to justify the conclusion that the epithelial hyperplasia or the connective tissue alterations represent true neoplasia.

### *Resolution*

When attempts are made on a purely morphologic basis to demonstrate a sequence of events in, or the processes of, disease, the results may be misleading. Nevertheless, the study of this large series of sections seems to justify tentative conclusions as to one form of retrogression of the lesion.

Desquamation of lining epithelium is frequently observed, but it is difficult to decide whether this is natural or due to fixation and preparation of the sections. However, when the technical work has been excellent it is reasonable to assume that epithelial desquamation, even of considerable degree, occurs naturally in gynecomastia. In only a

few cases did this produce denudation of the wall (Figs. 41 and 42). In 2 cases denudation was associated with local increase of inflammatory reaction. Whether the denudation caused the intensified exudation or resulted from it cannot be determined; however, such local accretions of exudate were not observed without denudation, a fact which indicates that exudation may be the primary event.

Healing of the lesion does not appear to be due to denudation or ulceration, but these processes may sometimes seem to precede resolution. In most instances resolution was brought about by reduction in size and epithelial content of the ducts so that there is a central solid mass of epithelial cells. These cells gradually disappear, leaving the hyaline band of basement membrane, which also disappears as healing advances (Figs. 43, 44, 45, and 46).

Accompanying these alterations in ductal epithelium, there is a notable increase in number of fibroblasts and capillaries (Fig. 35). This increment of what is really granulation tissue is fairly bulky and, as the process advances, there is condensation to a more mature type of white fibrous tissue (Fig. 47). In few cases in this series could the term cicatrix be justly applied nor was there hyalinization of the fibrous tissue.

Some degree of resolution was observed in 41 cases, but in none was the whole lesion entirely involuted. The areas of resolution were generally toward the outer part of a connective tissue mass and adjoining the fat; indeed, many were surrounded by fat. In these cases, other ducts showed epithelial proliferation, sometimes with active mitosis, and periductal or more widespread inflammation. The processes of proliferation and of resolution or involution go on side by side in the same lesion.

The process of involution in gynecomastia is probably not the only means by which the lesion retrogresses. Only by most careful examination of mammary glands which are known to have been previously the seat of gynecomastia can any conclusion be reached.

Fibroblasts, occasionally in great numbers, were often seen in the stroma. However, they were not associated with involution nor did they appear to produce mature connective tissue.

#### *Apocrine and Other Glands*

The sudoriferous coil glands, present in the sections, were tightly coiled as are such glands elsewhere (Fig. 48) and in no case appeared involved.

Apocrine glands, just under the musculature in the areolar region, were identified in 37 cases. They were more loosely coiled than the sweat glands and corresponded in detail to the description given by

Schiefferdecker<sup>28</sup> (Fig. 49). The cells were in a single layer, the cytoplasm acidophilic, and the nuclei basal in situation. Myoepithelium was usually seen more readily than in the sweat glands. When the gland was in active secretion, the outline of each cell was rounded toward the lumen and the cytoplasm contained many tiny, intensely acidophilic globules (Fig. 57). In later stages of secretion, acidophilic globules, often fused, appeared in the lumen, the cells were decreased in vertical diameter and the luminal bulge disappeared (Fig. 50). Rarely did the glands under the areola have the irregular outline of those in the axilla. In 8 cases, the apocrine glands were inactive.

No conclusive relationship could be established between the state of the apocrine glands and proliferative or inflammatory change in the associated mammary glands. In the controls the glands were found in both active and inactive states. It is unfortunate that few specimens included nipple and its associated structures.

Although, in cases in which there was dermatitis, infiltration of mononuclear cells sometimes appeared near sebaceous glands, there was no lesion of sebaceous or sudoriferous glands.

### *Dermatitis*

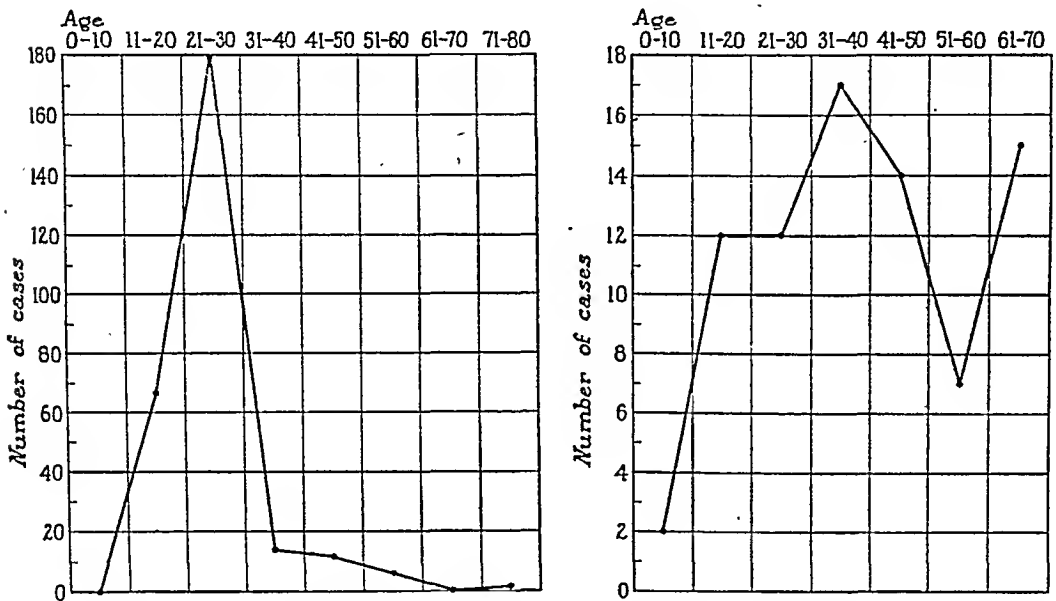
The skin of the normal male breast often contains a few lymphocytes and an occasional fibroblast in the papillary layer of the corium. In 14 cases of gynecomastia, about one-half of those in which skin was included in the specimen, the papillary layer was richly infiltrated with lymphocytes, plasma cells, and large mononuclear cells. The infiltration often extended well down into the reticular layer and was diffuse rather than perivascular or periglandular. In a few cases it was accompanied by dyskeratosis of the epidermis (Fig. 51). In 1 case a distinct crust appeared on the surface, but the section did not disclose actual ulceration (Fig. 52). It is reasonable to suppose that the subacute dermatitis was factitious rather than a part of the gynecomastia.

### DISCUSSION

*Incidence.* The Surgeon General's Office, through Col. E. R. Long, authorizes the statement that in the year 1943 the admission rate to Army hospitals for gynecomastia was 16 per 100,000 personnel. Webster<sup>10</sup> reported that in the Navy the rates for 1939, 1940, 1941, and 1942 were respectively 8.69, 7.40, 9.46, and 6.96 per 100,000. Gross enlargement of the breast is cause for rejection by the Navy; however, men with benign tumor of the breast or chest wall may be inducted by the Army provided the mass does not interfere with the wearing of a uniform or military equipment. In both services, clothing, straps across the chest, and psychic factors are probably about equal in bring-

ing the men to seek treatment. The difference in regulations governing acceptance could account for the divergence in hospital admission rates, although it is impossible to assess the influence of the interpretation of regulations by thousands of local induction centers. Nor is it necessary to assume that the admission rates necessarily represent the actual incidence among the personnel.

*Age.* Earlier in this paper the number of patients was shown in relation to various decades. The percentile distribution is about 16 in the second decade, 68 in the third, and 16 for the sum of the succeeding decades during which incidence falls rapidly. The general distribution approximates that of Army personnel, but statistical correction is not appropriate because of several factors.



Text-Fig. 1. Age distribution of cases. At the left, the distribution in the present series, set up in accord with Menville's "decades," for comparison with the distribution in his series, as shown at the right.

In a series, reported by Lewis and Geschickter,<sup>24</sup> of 95 cases in a large civilian hospital, the age was usually more than 40 years. The incidence in Menville's<sup>2</sup> 88 cases was highest between 31 and 40. In order to compare the figures in the 284 cases in the Army group with the smaller number in a civilian hospital, our distribution has been recalculated on the same basis as was Menville's. Text-Figure 1 shows the difference in "curves." The larger data show what is probably the more correct age incidence.

In 1868, Gruber<sup>29</sup> drew attention to the inconvenience and discomfort of soldiers with gynecomastia because of straps and tightly fitting uniforms. All 5 patients reported by Sullivan and Munslow<sup>22</sup> complained of aggravation of the lesion by the pack (pack-strap?). If this



applied to the series here reported, there might be an additional factor in determining age distribution.

The number of admissions for psychic reasons would be hard to establish. In a personal interview, an Air Forces surgeon told of a patient who insisted on operation because of ridicule in the barracks. It is probable that young men are especially subject to psychic trauma, but this cannot be verified, nor is it possible to say how many operations were performed on this account. Parachute straps do not irritate the breasts and the number of operations for gynecomastia in the Air Force is small, facts which may be used as evidence that the psychic factor is not important. Webster<sup>16</sup> indicated that all factors enumerated enter into operations on Navy personnel.

Gynecomastia has been reported in more advanced life; 2 patients in this series were respectively 76 and 77 years old. It has been observed in prepuberal life; for example, Ingleby<sup>27</sup> reported cases of boys 7 and 9 years old in which the lesion was unilateral and there was no indication of endocrine disturbance. It may be said that although gynecomastia may be observed at almost any time of life, it occurs most frequently in young men.

*Race.* Deaver and McFarland<sup>11</sup> intimated that gynecomastia is especially prevalent in African tribes, but the evidence is tenuous and based largely on the reports of lay travellers and explorers. Neither in other publications nor in this series is there any indication that gynecomastia is predominant in any one race.

*Breast Involved.* Although earlier writers and even an occasional recent one<sup>30</sup> stated that gynecomastia is usually bilateral, Menville<sup>2</sup> reported bilateral disease in only 12.8 per cent of 88 cases and Lewis and Geschickter<sup>24</sup> said it is most often unilateral. Geschickter<sup>20</sup> found it to be bilateral in 20 per cent of 108 cases. In this series, which is larger than either, bilateral involvement occurred in only 12 cases, or 4.6 per cent of the 262 in which location is given. Inequality was noted in only 1 case. Twice, bilateral lesions accompanied choriocarcinoma of the testis with metastases. Once they occurred in a patient with tuberculosis of the adrenals and Addison's disease which had been undiagnosed and untreated. One patient, 52 years old, with bilateral gynecomastia had been operated on for carcinoma of the bladder, but there is no note on the condition of the testes and there was no autopsy. There might have been testicular atrophy. The other 8 patients had no evidences of endocrine abnormality.

The figures in this or any other series may not be fully representative. Slight or even moderate equal enlargement of both breasts may not be noticed by the patient or the examiner, whereas enlargement of only one breast is likely to be obvious to both.

In the few instances of bilateral involvement, in which separate specimens were submitted from the two breasts, the lesions were microscopically identical. Thus, whatever causative factor or factors operated, the effect was the same in each breast.

*Duration of Complaint.* It may be said that in some instances the lesion appeared at or shortly after puberty, although in most it began in later life.

Jung and Shafston<sup>31</sup> made 1000 clinical examinations on 700 males between the ages of 9 and 45 years. They coined the term "subareolar node" to indicate a firm, disk-like mass under nipple and areola which they concluded appeared as an "integral part of the process of puberty." Even so, the nodes might be asymmetrical, were frequently tender and remained so for months. The involution was highly variable both as to promptness and completeness. In their series, 3 boys between 14 and 18 years had evident gynecomastia (without any signs of endocrine abnormality) in contrast to youths 17 years old with no remaining vestige of the node. Only 12 of 84 men in the 20.5 year group still had palpable nodules in one or both breasts.

It is evident that gynecomastia may be a continuation or augmentation of the pubescent node. It is equally evident that the lesion may arise in later life when the node can be assumed to have disappeared. There is no concrete evidence that gynecomastia is necessarily the sequel of the pubescent state. Adair<sup>30</sup> suggested that it may appear in the "male climacteric," but even if that state actually exists, there would be difficulty in finding support for the statement.

It is said that once gynecomastia develops it is permanent (Word and Reed,<sup>9</sup> Charache<sup>32</sup>) but the present study throws no light on the validity of that statement.

*Rate of Progress.* Judging from the 65 histories in which significant statements are recorded, progressive enlargement proceeded from a few weeks to several years, but most usually for a few months. Recurrent swelling was reported in two lesions lasting respectively 1 and 7 years. One enlargement present 11 years (no. 82515) grew rapidly in the last 4 months; another of 6 years' duration (no. 92295), in the last month. In case 82515 the number of ducts was markedly increased, whereas in case 92295 the increase was moderate. In both there was distinct epithelial proliferation, but not more than in other breasts that had been enlarged for years, and there was also moderate infiltration of mononuclear cells in the loose periductal tissue. Mitotic figures were observed in case 82515, but not in case 92295. The former also had a greater number of fibroblasts in the dense stroma. There was nothing seen in the microscopic examination which would separate these two

lesions clearly from others which lacked terminal acceleration of growth.

*Trauma.* The history of injury of some sort in 28 cases and of "possible injury" in 2 others is so vague, in general, as to be practically worthless. Various questions remain unanswered. Did gynecomastia exist before injury? Did injury accelerate the rate of growth? Did it simply attract attention to the lesion? Was it of enough magnitude to be significant? Did it act to provide receptors for endocrine stimuli? Examination of the sections gives no answer to these questions.

Various authors have referred to the effect of trauma, including Erdheim,<sup>3</sup> Word and Reed,<sup>9</sup> Webster,<sup>16</sup> Gill,<sup>18</sup> and Sullivan and Munslow.<sup>22</sup> The type of injury is highly variable. These writers made no important comments as to the relationship. Cheatele and Cutler<sup>5</sup> viewed with doubt the validity of injury as a cause. Impressive is the fact that neither in this series nor in the literature is there mention of injury in instances where outspoken endocrine disturbance appears to be causative.

*Subjective Symptoms.* Tenderness, dull ache, and pain of various intensities are frequent subjective symptoms. Erdheim<sup>3</sup> characterized the pain as "drawing" and as "lancinating." The fact that 94 patients complained of subjective symptoms suggests that they may be an integral part of the clinical picture. They seem unrelated to trauma, for only 2 of the patients who spoke of tenderness or pain gave a history of injury. In only a few did the swelling antedate the pain; on the other hand, in 1 case the pain was said to have preceded the swelling. Pain in the breast has been reported but is infrequent in gynecomastia associated with choriocarcinoma or following administration of stilbestrol; the more common complaint is a sense of fullness in the breast.

Sections from tender and painful breasts were reviewed especially with reference to inflammation and involvement of nerves. These did not differ in any essential from sections from breasts that gave no subjective symptoms. In neither group was there inflammatory or other involvement of nerves.

### *Microscopic Features*

The multiplication of ducts, the proliferation of epithelium, the formation of papillae and of intracanalicular masses of connective tissue observed in this study have also been described by others. There is general agreement among critical writers that acini and lobules are not formed. However, there is some difference of opinion and this confusion might be due in part to interpreting objective observations to fit the theory that gynecomastia is due to the action of substances with

the effects of estrogen and progesterone. Authors often attach too little importance to the alterations in the stroma. Ductal multiplication and epithelial proliferation may produce changes in particular parts of the gland which are like those observed in abnormal involution (chronic cystic mastitis) and its variations, but the lesion as a whole does not correspond to, nor is it in any way identical with, adenofibroma of the female. In fact, one case of adenofibroma of the female was included with the group of sections but it was readily distinguishable. To be sure, the individual changes in ducts can be found in various conditions in the female breast, with and without cancer, as described by Foote and Stewart,<sup>33</sup> but the association of the various ductal lesions is fairly characteristic. Furthermore, the picture is completed by the changes in periductal tissues and stroma as well as the infiltration of mononuclear cells. Even allowing wide variations, the over-all appearance differs significantly from that of other mammary diseases.

### *Secretion*

It is said casually by some writers that milk may be secreted in cases of gynecomastia. There are only a few references, however, in the literature to actual secretion of a fluid spontaneously or by expression. Zum Busch<sup>21</sup> reported that a "milky fluid" could be expressed in his case of gynecomastia associated with adrenal cortical tumor; Lissner<sup>34</sup> reported a watery fluid in similar circumstances. Secretion of a watery clear, a yellow, or a mucinous material in 3 of the 12 cases of gynecomastia reported by Erdheim,<sup>3</sup> was spontaneous in 2. Geschickter<sup>20</sup> found a discharge from the nipple in only 2 of his 108 cases. Del Castillo, de la Balze, and Reforzo Membrives<sup>17</sup> expressed a fatty liquid from enlarged breasts of a man with carcinoma of the lung, diagnosed as such only by roentgen study. Starr<sup>35</sup> said that in Basedow's case of gynecomastia in hyperthyroidism the swollen breasts secreted colostrum. John Hunter,<sup>36</sup> about 1790, referred to a man who suckled 8 children, but there is no satisfactory evidence that the man had gynecomastia nor that he secreted milk; he was not a hermaphrodite. Gynecomastia was not reported in Hänel's<sup>37</sup> case. Numerous authors have referred to the case of the Zulu chief, Chenwayo, whose name is variously spelled. This man was about 55 years old, weighed about 400 pounds, had 40 wives and, as the story has grown, he nursed more and more children. As a matter of fact, the original report is based on a picture exhibited by a lay travel-lecturer. The statement by Major Shufeldt<sup>38</sup> is: "It is stated that he is the father of more than one hundred children, and the supposition is that he had nursed some of them." This is hardly acceptable evidence.

Of interest was the observation of Roth<sup>39</sup> in a case of acromegaly. The breasts were not enlarged but the nipples projected and the areolae were enlarged. On moderately strong pressure a milky fluid was extruded in which were fat globules but no leukocytes or colostrum corpuscles; it was alkaline and contained 8.5 per cent sodium chloride as well as casein, fats, lipoids, and lactose. There were no data on microscopic examination of the mammary tissue.

There is no satisfactory evidence that the mammary gland of gynecomastia secretes milk. Experimental evidence is suggestive but not conclusive. Van Heuverswyn, Folley, and Gardner<sup>40</sup> found that sex hormones, both male and female, excite a secretory response in spayed female monkeys, but the exact nature of this secretion is not stated. Frazier and Mu<sup>41</sup> administered over a long period the estrogenic substance from the urine of pregnant women to male rabbits. "Milk" could be expressed from the nipples; the fluid from 6 animals became thick and white, from 2 it remained thin and watery. The animals willingly fostered young and 2 suckled them. Three animals, 8 days old, survived 5 to 6 days, 1 to 2 days longer than controls. None of this proves that the material was milk. Brownell, Lockwood, and Hartman<sup>42</sup> treated adrenal cortical extract so as to produce a substance which they called corticolactin. Adrenalectomized mother rats given this material raised 64 and 41 per cent of their young as compared with 2 and 13 per cent raised by those given cortin alone and 72 per cent raised by normal controls. This work has not been confirmed and some investigators regard the evidence that corticolactin is a special hormone as tenuous. Goldzieher<sup>43</sup> suggested that the effects may be due to estrogenic substances in the extract. The appearance of witch's milk is probably due to maternal hormones. No definite evidence exists that milk is produced by these experimental methods.

That secretion occurs in gynecomastia is undoubted. In the 284 cases in this series, albuminous as well as mucinous secretion was noted in the sections; and in the duct of the nipple in 1 case a material like colostrum was found. In no sense can these secretions be thought of as peculiar to the mammary gland as compared with other glands.

### *Inflammation*

Only a few writers have reported the presence of mononuclear or other cells in the loose periductal tissue or in the dense stroma. The fact that polymorphonuclear leukocytes are sometimes associated suggests that such cells are a part of inflammation. Nevertheless, there may be a reversionary factor, to judge from the reports of von Eggeling, Hoepke, and Kolmer<sup>44</sup> that in fetal life hematopoietic centers may occur near tubules and in the connective tissues but disappear by the

time of birth. These centers contain immature erythrocytes, eosinophilic myelocytes, cells with the structure of plasma cells, others of various forms with neutrophilic or basophilic granules in the cytoplasm, giant cells and histiocytes, all probably formed from adventitial cells of the blood vessels. However, the cells in gynecomastia are like those seen in interstitial inflammation in other organs where there are no pre-existent hematopoietic centers. The subsequent development of granulation tissue favors the view that the cells are exudative and the process inflammatory.

Similar infiltration into the stroma may be perivascular. In this situation granulation tissue was not observed, but fibroblasts were fairly common.

### *Neoplasia*

In the material studied there was no true neoplasia, either benign or malignant; neither was there clear evidence in the literature that gynecomastia may develop into cancer. Retraction of the nipple may occur, especially when cysts are present or when concurrent inflammation has caused adherence to surrounding structures. Occasionally confusion may result from enlargement of the axillary lymph nodes; however, Erdheim<sup>3</sup> has pointed out that this process is only simple hyperplasia.

Word and Reed<sup>9</sup> stated that "carcinoma and sarcoma have both been observed to develop subsequent to gynecomastia and fibroma of the male breast." Gilbert<sup>45</sup> said that 9 (19 per cent) of his patients with carcinoma of the male breast had gynecomastia. Cheatle and Cutler<sup>5</sup> reported 1 case of gynecomastia with malignant papilloma. Geschickter,<sup>20</sup> however, found no malignant disease in 108 cases of gynecomastia. Ewing<sup>14</sup> stressed the importance of chronic cystic mastitis as a precursor of carcinoma of the breast in the female, but did not suggest that gynecomastia leads to cancer.

The reports of malignant changes in gynecomastia are evidently based on the assumption that any enlargement of the male breast is gynecomastia. No consideration is given to the possibility that enlargement may be due to the neoplasm rather than to the phenomena which constitute gynecomastia. The fact that Geschickter<sup>20</sup> noted no neoplastic disease in his series and that none was found in this larger one must indicate that neoplasia, if it ever follows gynecomastia, is exceedingly infrequent.

### *Resolution*

There is a general impression among clinicians that once gynecomastia is established it does not naturally undergo retrogression. The material studied in this report gives no evidence one way or the other, but the assumption of others is probably valid. Nevertheless, focal

retrogression or resolution does occur, but there is no ground for stating that it affects the mammary gland as a whole. Indeed, the fact that such resolution is concurrent with active progress of the disease leads to the contrary view. Such foci of resolution are rarely seen in other lesions of the breast, except, as described by Muir,<sup>40</sup> in the intraductal carcinoma of the mammary gland associated with Paget's disease of the nipple.

### *Apocrine Glands*

The apocrine glands may be active or inactive quite independently of the proliferative or inflammatory condition of the associated mammary gland. If endocrine activity plays a part in gynecomastia, it is impossible to correlate it with the apocrine glands.

"Apocrine epithelial proliferation" (so-called pale glands) is seldom observed deep in the mammary gland in gynecomastia. In the material reported herein, pale glands were not large enough to be visible grossly, as described by Foote and Stewart<sup>33</sup> in various diseases of the female. The acinic structure is different from that of true apocrine glands. Although Ewing<sup>14</sup> suggested that apocrine glands are derived from the sudoriferous coil glands said to accompany mammary ducts, it seems more probable that they represent a change in proliferated ductal epithelium, which may be metaplasia or a reversionary process to be explained by the embryonal origin of mammary and sudoriferous epithelium. At any rate, these altered ducts differ in situation and structure from the subareolar apocrine glands.

### *Dermatitis*

Subacute dermatitis, sometimes with ulceration and crusting, is fairly frequent. It is not an essential part of gynecomastia, but may be due to irritation or may possibly be factitious. Ewing<sup>14</sup> spoke of chronic eczema as a contributing factor to mammary carcinoma of males, but the lesion seen in this series would not qualify as eczema.

### ETIOLOGY

It is probably true that, as Erdheim<sup>3</sup> said, in most cases gynecomastia is the primary disease; in some, however, the lesion is obviously secondary to other conditions, and in still others presumptive causes are mentioned without proof that they are effective.

Heredity has been suggested as a factor. Savitsky<sup>47</sup> reported a patient, 21 years old, with massive pendulous breasts, whose father, a brother, and a cousin also had large breasts. The exact nature of the lesion was not investigated. Hutchinson's<sup>48</sup> case of the son of an acromegalic woman was certainly pseudogynecomastia. I agree with

Cheatle and Cutler<sup>5</sup> that there are no satisfactory records of hereditary influence. Among the cases of gynecomastia so far reported there is nothing to indicate a hereditary or constitutional affection of endocrine glands.

### *Endocrine Factors*

*Testes.* In 1894, Williams<sup>15</sup> attributed gynecomastia to various diseases of the testis, to atrophy, to injury, and to excision of these gonads. According to Menville,<sup>2</sup> Schmit had reported the lesion associated with orchitis in 1891, and Martel with varicocele in 1893. Subsequently, Weber<sup>4</sup> asserted that gynecomastia might be the sign in adults of destruction of the testes, and attributed gynecomastia to lesions of the testis except those in which the interstitial cells are spared. Tellgmann's<sup>49</sup> widely quoted case of gynecomastia on the same side as a testis which had been crushed and destroyed was almost certainly pseudogynecomastia, especially as he stated specifically that glandular tissue could not be palpated. Cheatle and Cutler<sup>5</sup> gave credence to the view that disease and atrophy of the testis are followed by gynecomastia. Ilabaca Leon<sup>8</sup> associated the lesion with various genital defects. Webster<sup>16</sup> said that in a few castrated males and in some with prostatic enlargement, gynecomastia was associated with endocrine imbalance. Richardson<sup>50</sup> suggested that testicular atrophy may cause reduction in amount of male sex hormone.

In none of these reports is concrete evidence offered on the basis of actual assays of endocrine secretions.\* Furthermore, in many instances no distinction has been made between the fatty breasts of pseudogynecomastia and true gynecomastia. Pratt<sup>51</sup> pointed out that the breasts may share in obesity following bilateral orchiectomy in prepuberal life and occasionally in adults. Zondek<sup>52</sup> said that eunuchs may have fatty breasts. Lewin<sup>23</sup> stated flatly that bilateral excision of the testes does not produce gynecomastia. Kriss<sup>1</sup> maintained that neither in man nor in animals is there anything to support the view that the testes influence the development of the breast. Erdheim<sup>3</sup> excluded nonneoplastic testicular lesions as causative of gynecomastia.

Nevertheless, there is experimental evidence of some relation between mammary gland and testes. Richardson<sup>50</sup> reported that castration prevents the normal development of the breast in male rats at puberty. Deanesly and Parkes<sup>53</sup> showed that certain androgens may produce some of the manifestations of feminization but did not refer

\* Throughout the following discussion, emphasis is laid on the importance of assays of hormones or their products. This is with full recognition of the sources of error and the fact that such assays do not necessarily indicate what happens to the hormones in the body. Yet the assays are the nearest objective approach to the problem.



specifically to gynecomastia. Selye, McEuen, and Collip<sup>54</sup> described slight development of the mammary glands in gonadectomized female rats when testosterone benzoate was administered. Van Heuverswyn, Folley, and Gardner<sup>40</sup> reported that androgens induce growth of the main ducts of the mammary glands of male guinea-pigs, of immature male and female rats, and of spayed virgin rats, but that the effect of different androgens varies. There is also a body of evidence to indicate that gynecomastia may be due to estrogens. If that be so, another effect of estrogenic substances is atrophy of the testes. Dunn<sup>55</sup> reported atrophy of the testes in "hypersexual" males following administration of stilbestrol, but testicular tissue was not examined microscopically. McEuen, Selye, and Collip<sup>56</sup> showed, however, that the mammary gland does not develop in male rats castrated in early life and, further, that castration of adult males leads to involution of the already developed breast. It is well known that in Cushing's disease and various tumors of the endocrine glands the testes become atrophic, usually as a result of deterioration of the seminiferous tubules while the interstitial cells may be reduced, increased, or of about the normal number. Frazier and Mu<sup>41</sup> found that administration of pregnancy urine to rabbits led to atrophy of the testes, but this had no microscopic confirmation. Bonser and Robson<sup>57</sup> found that the effects of prolonged administration of estrogens differed for three strains of mice. In CBA and R III strains, the testes became atrophic and spermatogenesis ceased, but in the Strong A strain, spermatogenesis persisted in spite of marked hyperplasia of interstitial cells. These and other reports support the view that estrogens may produce testicular atrophy, but their effects are not uniform.

The relation of testicular disease, other than neoplasia, to gynecomastia is not clear. Certainly in man there are too many cases in which the testes are morphologically and functionally normal to justify the belief that atrophy or destruction of the testes is a significant cause of gynecomastia.

*Tumors of the Testis.* The tumors of the testis concerned in gynecomastia fall into two great groups: (1) teratoma and choriocarcinoma, and (2) interstitial cell tumors.

Lewin<sup>23</sup> quoted Heidrich as reporting gynecomastia in 8 of 140 patients with teratoma testis, and Ferguson in 5 among 117. It has been difficult to determine how many of these are cases of choriocarcinoma, how many of embryonal carcinoma, and so on through the list of testicular tumors. Kriss<sup>1</sup> considered testicular tumors the most frequent neoplastic causes of gynecomastia, but this is not proved. In my Army series testicular tumors found were 2 embryonal carcinomas,

4 malignant teratomas, and 1 choriocarcinoma. In some of the cases in which teratoma was diagnosed, the tumor is undoubtedly choriocarcinoma. An example of the confusion of nomenclature is the report by Craver and Stewart<sup>58</sup> of a choriocarcinoma under the name teratoma, undoubtedly due to Ewing's claim that choriocarcinoma and embryonal carcinoma represent one-sided development of a teratoma.

Cairns<sup>59</sup> observed gynecomastia in 2 of his cases of neoplasm of the testis identified as teratoma, and found that the enlargement of the breast coincided with the development of metastases. He cited cases of Cooke, of Warthin; and of Malloch, all of which were choriocarcinoma; also a case of Garbarini, in which the gynecomastia subsided after removal of the tumor. Bonn and Evans<sup>25</sup> reported a case of extragenital choriocarcinoma in which one breast became enlarged before the other. In this case the Friedman test was positive and the assay for prolactin showed more than 10,000 and less than 100,000 units. Perhaps more instructive are the assays in the case of extragenital choriocarcinoma with bilateral gynecomastia reported by Laipply and Shipley,<sup>60</sup> as follows:

*Hormone Assays on 24-Hour Urine Specimens*

	Gonadotrophin Chorionic Type in Mouse Units	Estrogen Internat- ional Units	17-Keto- steroids in mg.	Pregnane- diol in mg.
Case of Laipply and Shipley	300	750+	27	2+
Normal male	0	75—	25	0.1
Normal female	0	50-300 (ovulation)	12	45 in luteal phase
Teratoma testis	Elevated	Not known	No change	Not known
Late pregnancy	6,000	10,000	No change	30-40
Choriocarcinoma of uterus	200,000 to 1,000,000	Elevated	No change	Not known

The increase of chorionic gonadotrophin,\* of estrogen, and of pregnanediol is apparent, but there is no significant change in 17-ketosteroids. No particular influence of gonadotrophins on the breast is known, but in certain animals estrogens stimulate proliferation of ducts, and progesterone, when acting with estrogens, the acini. In this case ductal proliferation was marked. The slight increase in pregnanediol, as an index of progesterone, did not lead to acinus formation, but the level may have been too low for such an effect to be expected. In Bonn and Evans'<sup>25</sup> case, the high prolactin may have operated through the medium of other factors. In Craver and Stewart's<sup>58</sup> case with high content of follicle-stimulating hormone, gynecomastia is not mentioned.

Bilateral gynecomastia occurs in cases of tumors of the testis of the

\* The spelling now widely used by endocrinologists has been adopted in this paper.

general order of choriocarcinoma and probably in other tumors, presumably because of increase in estrogenic substances. As yet there is no clear indication that progesterone is effective nor can gonadotrophins be directly incriminated, especially since in Laipply and Shipley's<sup>60</sup> case the increase was in chorionic gonadotrophin rather than that produced by the pituitary body.

Gilbert<sup>61</sup> analyzed 123 cases of what he called the syndrome of choriogenic gynecomastia. These included cases of choriogenic hyperplasia, primary testicular choriocarcinoma, extragenital choriocarcinoma, apparently misdiagnosed testicular tumors, and teratoid tumors of the testis with choriocarcinomatous metastases. Although endocrine assays were made, usually incomplete, in 46 cases, he concluded that "choriogonadotrophic hormones and folliculin exert a stimulating effect on the pituitary, producing pseudopregnancy changes with hyperplasia of the breast and accessory sexual organs." Shimkin<sup>62</sup> pointed out, however, that, at least in mice, chorionic gonadotrophin does not affect the mammary gland.

The relationships of the interstitial cells are not clear. The association of atrophy of the testes with gynecomastia is not frequent and little information is gained without microscopic examination. Choriocarcinoma may be accompanied by atrophy of the testes. In Craver and Stewart's<sup>58</sup> case the seminiferous tubules were atrophic and the interstitial cells hyperplastic, as has been true of my cases. They quoted the cases of de Vries and of den Hartog in which, however, there was no hyperplasia of interstitial cells. Richardson<sup>59</sup> suggested that testicular atrophy leads to absolute reduction in amount of male sex hormone and that the imbalance with the relatively large ratio of estrogens results in stimulation of the mammary glands. The possibility that balance may be preserved by production of androgens in the adrenal cortex is given no consideration, and neither is the demonstration by Van Heuverswyn, Folley, and Gardner<sup>40</sup> that androgens may of themselves stimulate mammary growth. In this connection Klinefelter, Reifenstein, and Albright<sup>63</sup> reported cases of gynecomastia, aspermatogenesis without a-leydigism and increased excretion of follicle-stimulating hormone. They concluded that the gynecomastia in these cases was not due to hyperestrinism nor to androgen alone, but possibly to a combination of androgen and lack of inhibin. Heller and Nelson<sup>64</sup> agreed that increased amounts of gonadotrophic hormone, and low normal or reduced quantities of androgen and estrogen might bring about gynecomastia, but pointed out that there is no proof of the existence of inhibin. Furthermore, they found that gynecomastia does not always occur in the syndrome.

Hunt and Budd<sup>65</sup> reported the case of a white man, 42 years old, with enlarged, painful breasts, impotence, and an interstitial cell tumor of the testis. After removal of the tumor the enlargement of the breasts receded and libido was restored. The urine contained 1000 units of luteinizing hormone per liter, but other determinations were not reported. The cells of the tumor contained mitochondria-like granules, but the crystalloids of Reinke\* were not found. This case raises the question common to all tumors of the testis designated as interstitial cell, namely, whether they are derivatives of interstitial cells or of adrenal cortical inclusions. The fact that the interstitial cells normally contain refractile globules of lipids, brown pigment, and the crystalloids of Reinke does not mean that these would occur in neoplastic cells, but if they are found there would be no reasonable doubt of the cytogenesis. In their absence, however, the cells cannot positively be distinguished from neoplastic cells derived from adrenal cortex. Endocrine studies have not been conducted to a sufficient extent to permit of conclusions. In Craver and Stewart's<sup>58</sup> case of obvious interstitial cell hyperplasia without gynecomastia, follicle-stimulating hormone was present in excess, whereas in Hunt and Budd's<sup>65</sup> case the increase was in luteinizing hormone. From the functional point of view the tumor reported by Hunt and Budd might possibly have been an adrenal cortical neoplasm.

Monaschkin's<sup>66</sup> case of testicular tumor with gynecomastia is repeatedly cited as an example of interstitial cell tumor, but it may well have been an embryonal carcinoma. He cited Benda as having offered the speculation that the tumor was derived from embryonal cells of feminine type (origin not further specified) which favor the growth of mammary glands and lead to testicular atrophy. There is little doubt that in certain strains of animals the prolonged administration of estrogens may lead to the development of testicular lesions which have many of the features of interstitial cell tumors, as illustrated in the work of Bonser and Robson.<sup>57</sup> Bonser and Hawksley<sup>67</sup> compared the lesions in 2 human cases with the experimental material from mice and concluded that they were identical, but too little analysis of endocrines was done to confirm this opinion. The Aschheim-Zondek test, performed in only 1 case, was negative.

The influence of interstitial cells of the testis upon gynecomastia and the converse is far from clear. It cannot be proved that interstitial cell tumors are accompanied by gynecomastia until a positive means is

\* Reinke, F. Beiträge zur Histologie des Menschen. Ueber Krystalloidbildungen in den interstitiellen Zellen des menschlichen Hodens. *Arch. f. mikr. Anat.*, 1896, 47, 34-44.

established to differentiate in man between tumors of that order and those which may be derived from adrenal cortical rests.

*Ovary.* Large breasts may be observed in hermaphrodites with ovary and ovotestis. Although these persons may have the secondary sexual characters of males, they are examples of true intersex and the large breasts are attributable to ovarian function. This is not gynecomastia. In cases of intersex the adrenal cortex may be notably hyperplastic. Shimkin<sup>62</sup> reviewed fully the work of Loeb and Lathrop concerning the influence of ovaries on growth of the mammary gland and the development of tumors, but this has no direct bearing except as concerns the effects of estrogen.

*Adrenal Cortex.* The hormones of the adrenal cortex are known to influence secondary sexual characters. Enlargement of the breasts in males with Cushing's disease or syndrome is such that, as Glass and Bergman<sup>68</sup> said, "gynecomastia may now be included in the category of adreno-genital syndrome." They reported that in virilized females, the ratio of androgen to estrogen was increased but that in 2 men with gynecomastia, the opposite was true. Although Parkes<sup>69</sup> favored the importance of ratio, Goldzieher<sup>43</sup> seemed doubtful.

Several cases of cortical tumor of the adrenal with gynecomastia have been recorded. Bittorf's<sup>70</sup> case of a man, 26 years old, is somewhat dubious because of an inadequate report on the tumor. Mathias'<sup>71</sup> case of enlarged breast in a female, 3 years old, with adrenal cortical tumor is suggestive, but the enlargement was not gynecomastia even though the girl showed many signs of virilism. The patient of zum Busch<sup>21</sup> with painless gynecomastia and secretion of a milky fluid was a man, 27 years old, who had carcinoma of the left adrenal cortex and metastases. Weber<sup>4</sup> included this case in one of his papers. Holl<sup>72</sup> reported 2 cases. One of bilateral gynecomastia and tumor in the region of the left adrenal, which Beitzke called "hypernephroid," was that of a boy aged 15. The other of bilateral gynecomastia and atrophy of the testis was of a man, 44 years old; removal of a tumor from the cortex of the left adrenal resulted in restoration of sexual function and regression in size of breasts. Simpson and Joll's<sup>73</sup> patient was a man, 34 years old, with bilateral gynecomastia and reduced size of genitalia. After the cortical adrenal tumor was removed it recurred. Assays of hormones at this time gave evidence that estrogens were increased and androgens were only slightly increased, chiefly D<sup>3:5</sup> androstadiene-17-one. Broster and associates<sup>74</sup> reported the case of a man, 38 years old, with bilateral enlargement of breasts, adrenal cortical carcinoma, and an excess of estrogen in the urine. Lisser's<sup>34</sup> patient was a man, 33 years old, with markedly enlarged breasts, firm but not tender. A

malignant tumor near the kidney was called an adrenal cortical tumor, although, since neither the kidney nor adrenal was involved, it was supposed that the neoplasm was derived from an adrenal rest. Unfortunately there were no endocrine assays. "A watery fluid could be expressed at times from the breast." This was interpreted as lactation!

There is sufficient evidence that hyperplasia of the adrenal cortex as well as cortical tumors, benign or malignant, may be accompanied by gynecomastia. Just what hormone or hormones may operate is not clear, but it is unlikely that neutral 17-ketosteroids in the urine are increased. For example, Warren<sup>75</sup> found normal values for 17-ketosteroids in 1 case of gynecomastia. Zondek<sup>52</sup> referred to the heterosexual function of adrenal cortex, as indicated in virilization of females and rare cases of suggestive feminization of males; exact correlation awaits more assays of hormones. Of interest is Allen's<sup>76</sup> observation that tumors of the adrenal cortex may develop in mice after early ovariectomy and that there is evidence that the tumor secretes estrogen; in susceptible strains of mice mammary carcinoma may arise.

Reference has already been made to experiments in which the breasts of animals have been stimulated with androgenic substances. It is noteworthy that in the work of Van Heuverswyn, Folley, and Gardner,<sup>40</sup> the best growth of the breast followed administration of desoxycorticosterone acetate and androstenediene. The reports on treatment of Addison's disease in man by cortical extracts are also suggestive. Edwards, Shimkin, and Shaver<sup>77</sup> observed that a man of 35 years, with Addison's disease due to cortical atrophy of the adrenals, had enlarged, tender breasts after daily injections of eschatin for 3 months. Treatment was stopped and the breasts regressed only to grow again when treatment was resumed. The same sequence was reported in Lawrence's<sup>78</sup> case, in response to desoxycorticosterone acetate. In Raleigh and Philipsborn's<sup>79</sup> case of a man aged 40 with Addison's disease, the right breast became enlarged after 2 years of treatment with desoxycorticosterone acetate. The unilateral enlargement persisted without involving the opposite breast, an exception to the general rule of bilateral enlargement in unquestionable endocrine cases. It is interesting, but does not bear directly on the problem, that a 30-year-old patient in this series with bilateral gynecomastia had Addison's disease with tuberculosis of the adrenals, but since this was not discovered until autopsy, the patient had had no hormonal treatment of any kind.

Gynecomastia may result from administration of two extracts from the adrenal cortex, eschatin and desoxycorticosterone acetate, but this is rare. The significance of this observation is attested to by reduc-

tion in size of the breasts upon stopping treatment and subsequent enlargement when it is resumed, as well as by the effects of cortical hormones on experimental animals. Whether gynecomastia in cases of cortical tumors is to be explained in the same way is not yet established, but in the cases of Simpson and Joll<sup>73</sup> and of Broster and associates<sup>74</sup> the output of estrogen in the urine was increased. Thus there remains a question as to whether adrenal cortical hormones or estrogens were more significant, or whether hormonal imbalance was important.

*Pituitary Body.* Although several authors speak of gynecomastia in disease of the pituitary body, there is little in the way of documentation. If Cushing's disease be primary in the pituitary gland, certainly gynecomastia has been observed, but in practically all cases the adrenal cortex is hyperplastic. Roth's<sup>39</sup> case of acromegaly, in which the breasts secreted a milky fluid, was without gynecomastia. Moehlig's<sup>80</sup> patient, 52 years old, had had bilateral enlargement of the breast for 2 years. Autopsy limited to the head disclosed a chromophobe adenoma of the pituitary gland. The man was fat, had adenomatous goiter, a female type of escutcheon, and atrophic testes, but there was no indication of true gynecomastia. There may have been other lesions in the body; certainly there was no proof that gynecomastia was due to the pituitary tumor.

Heinbecker<sup>81</sup> expressed the opinion that in Cushing's disease the lesions of the pituitary gland are not primary, but are due to hypothalamic dysfunction. In a discussion of precocious puberty, Weinberger and Grant<sup>82</sup> formulated the hypothesis that lesions in nerve tracts between hypothalamus and pituitary body interrupt inhibitory stimuli to the latter, thus releasing gonadotrophic hormones. If this were the case, androgenic or estrogenic hormones might be increased in amount. Lactogenic hormones are evidently produced by the pituitary body, but there is no proof that they induce morphologic hyperplasia. Except in Cushing's disease there is no regular association between pituitary lesions and gynecomastia.

Experimental study is handicapped because prolactin is the only pituitary hormone isolated in crystalline form and there is no evidence that it stimulates growth or formation of tumors in the mammary gland.<sup>62</sup> Nevertheless, the hypophysis may play a part, because, at least in mice, hypophysectomy inhibits the proliferation of the mammary gland induced by estrogens.

*Thyroid.* Starr<sup>35</sup> found in the literature 8 cases with associated hyperthyroidism and gynecomastia, to which he added 2. He referred to Basedow's case in which the enlarged breasts are said to have

secreted colostrum. Starr's first patient had bilateral gynecomastia with increased metabolic rate. For technical reasons only hemithyroidectomy was performed and it was followed by reduction of basal metabolic rate and recession in the size of the breasts. With a subsequent rise in basal metabolic rate the left breast again became enlarged. Only the right breast of the second patient was enlarged. After thyroidectomy, the basal metabolic rate which had been increased, fell to minus 3 and the right breast became normal in size. Starr referred to experiments of Weichert and Boyd, who found that the feeding of thyroid to pregnant rats resulted in greater hypertrophy of the breasts than was true of normal controls. Petersen, Knodt, Ludwick, and Pomeroy<sup>83</sup> said that "the rôle of the thyroid in mammary gland development and lactation is still controversial." The general impression is that thyroid substance is not effective in producing mammary tumors.

The augmented metabolic rate apparently was accompanied by gynecomastia, but without hormone assays other factors cannot be excluded. The experiments cited throw no light on the mechanisms concerned. Thus the effects of endocrines other than the thyroid have not been ascertained. The influence of the thyroid upon the cellular structure of the pituitary gland and the stimulation of lactation by thyroxin are well known, but they have no direct bearing on the hyperplasia of gynecomastia.

### *Hepatic Cirrhosis*

Edmondson, Glass, and Soll<sup>84</sup> have referred to the association of atrophic cirrhosis, ascites, testicular atrophy, and gynecomastia as the Silvestrini-Corda syndrome. They said that 65 cases had been reported in foreign literature but none in English. They reported 8 additional cases, in which gynecomastia developed late in the disease after ascites had occurred. The changes in the breasts were principally proliferative like those due to administration of estrone to animals. They cited Sevringhaus, Israel, and others who have shown that the liver acts normally to destroy estrogen, and cited 2 of their own cases in which excretion of total estrogen in the urine was augmented and 2 others in which free estrogen was increased. Schiller and Pincus<sup>85</sup> reported that in rats, extirpation of lobes of the liver interferes with the conversion of estrone to estriol. Although Cantarow, Paschkis, and Rakoff<sup>86</sup> expressed doubt that the liver inactivates estrogens *in vivo*, the demonstration of excess excretion in the urine of actual cases of cirrhosis makes it seem probable that even the rare case of gynecomastia associated with hepatic cirrhosis may well be due to the pro-



longed action of estrogenic substances. The fact that gynecomastia has not been reported in acute hepatic disease may be because of too short an action or because of the absence of the dietary deficiencies which play a part in development of cirrhosis. The testicular atrophy may be like that which accompanies chronic disease in general or may be due to the effects of estrogen.

### *Prostatectomy*

Cheatle and Cutler<sup>5</sup> said that there are numerous instances of gynecomastia in older men following prostatectomy. Oppenheimer<sup>87</sup> reported 1 case of a man, 64 years old, with bilateral gynecomastia which began 3 months after prostatectomy, and another, 48 years old, with bilateral gynecomastia "several" months after operation. He offered the theory that cicatrization of the ejaculatory duct is, in effect, a Steinach operation. Mann<sup>88</sup> reported the case of a man, 70 years old, who acquired bilateral gynecomastia 2 years after prostatectomy. He cited Kandellon as reporting 2 cases, both in men of 70; in one, gynecomastia appeared in the right breast 1 month after operation and disappeared in a year; in the other, the right breast became enlarged 3 months, and the left 4 months postoperatively. He advanced the theory that the prostate may elaborate an internal secretion, but this is pure speculation.

The reason that gynecomastia appears in old men, according to Cheatle and Cutler,<sup>5</sup> is that they are the people who have had prostatectomy. One of Oppenheimer's<sup>87</sup> patients was 48 years old, which suggests that prostatectomy is the important factor. I have found no record in which the lesion followed transurethral resection. Until endocrine assays are made, no conclusions are possible concerning prostatectomy as a causative factor.

### *Pulmonary Disease*

Del Castillo, de la Balze, and Reforzo Membrives<sup>17</sup> reported 3 cases of bilateral, but unequal, painful enlargement of the breasts. In all, sexual potency was reduced and primary carcinoma of the lung had been diagnosed clinically, although in only 1 was it proved by autopsy. These authors cited Secco's 5 cases of unilateral gynecomastia with lesions in the thorax: 2 were pulmonary abscess; 1 was suppurating hydatid cyst; 1, spontaneous pneumothorax; and 1, bullet wound in the left infraclavicular region. These reports prove nothing, but do suggest that men with gynecomastia are not immune to infection, neoplasm, or injury, and that the converse is equally true.

*Estrogenic Hormones*

The natural estrogenic substances used experimentally include estrone, estradiol, estriol, equilin, equilinin and their benzoates. The synthetics are particularly diethylstilbestrol and its dipropionate, and also triphenylethylene; these do not contain the steroid nucleus but are highly estrogenic. Estrogens excite development of ducts. In some species acini and lobules are produced, but in others progesterone as well is required for the growth of acini.

Lewis and Geschickter<sup>24</sup> injected Amniotin-Squibb into male monkeys and found that the best results were obtained when small doses were given over a long time. Bilateral enlargement of the breasts occurred and microscopically the changes were like those of human gynecomastia. They reported that castration favored regression of the lesion. Chorionic gonadotrophin from pregnancy urine produced similar but less marked changes, but it is not indicated that estrogens were removed from the extract. Gardner and Van Wagenen<sup>89</sup> reported varying results in different species. Estrogens alone gave rise to full mammary development in guinea-pigs. In rabbits, estrone induced growth of ducts, but glands appeared only when extract of corpus luteum was added. In both male and female mice, prolonged administration of estrogens seemed to lead to formation of localized nodules of alveoli. They cited Allen<sup>76</sup> who, by administration of estrin, had partially restored the atrophic breasts of ovariectomized monkeys, and Turner and Allen who stimulated ductal growth in male monkeys and some alveolar development in females by the same treatment. They referred to Aberle who found that young female monkeys gave no response to theelin, whereas growth of ducts was accelerated in young males. Gardner and Van Wagenen reported that the response to treatment with theelin, theelol, or hydroxyestrin benzoate was essentially the same; in male monkeys each induced proliferation of ducts and development of acini.

That these results are directly applicable to man is open to some question and the same is true of the generalization concerning the action of estrin and progesterone. Dunn<sup>90</sup> reported that when estradiol benzoate was used to treat migraine in males the breasts became enlarged, but he said nothing about ducts and acini.

Diethylstilbestrol, 4'4'-dihydroxy-a-b-diethylstilbene, a synthetic preparation with estrogenic activity, has been studied experimentally and has been used in the treatment of the metastatic lesions of prostatic carcinoma. Smith and Smith<sup>91</sup> found that stilbestrol is 100 times as active as estrone in bringing about release of gonadotrophic factors in

mature male rats. These responses are not identical with those to estrone, because in castrated male rats the administration of progesterone depresses the activity of estrone but not of stilbestrol. Smith<sup>92</sup> reported on the lack of identical action by the two substances. In experimental animals triphenylethylene has much the same effect as stilbestrol.

Nevertheless, stilbestrol produces essentially the same changes in the breast as those which may be attributed to natural estrogens. Dunn<sup>93</sup> reported gynecomastia resulting from the treatment of a "sex criminal" with stilbestrol. Moore, Wattenberg, and Rose<sup>26</sup> gave a fuller account of a case of prostatic carcinoma since it included reports of biopsies of the enlarged and sometimes painful breasts before and after treatment with stilbestrol. The mammary ducts became elongated and early showed budding. The epithelium proliferated, producing additional lining layers of the ducts, even to the point of filling their lumina. There was expansion of the connective tissue stroma, which appeared edematous because of pale staining, and later the fatty tissue increased. The photomicrographs indicated inflammation. The authors seemed to regard the process as a chronic cystic mastitis. There were no endocrine assays.

The same authors<sup>26</sup> stated that there were no indications of cancer, nor was there evidence in the literature that stilbestrol produces cancer of the human breast. Although it is true that this does not occur regularly, the development of mammary cancer after administration of natural estrogens in animals is known to be a possibility. The work of Lacassagne,<sup>94</sup> of Burrows,<sup>95</sup> of Bonser,<sup>96</sup> of Gardner, Smith, Allen, and Strong,<sup>97</sup> and others, indicates that administration of estrone benzoate or of ketohydroxyestrin may be followed by mammary cancer in mice. Transplantation of the cancer is reported by Bonser and by Gardner and his associates. Burrows found several instances of hypertrophy of the mammary epithelium in noncancerous breasts and Bonser reported branching of ducts and distention of lumina in which were albuminous material and epithelial debris; these changes, together with a number of small cysts, were reminiscent of chronic cystic mastitis.

By means of biopsy and post-mortem examination, Eisen<sup>98</sup> studied the process in male rats treated with crystalline estrin (estradiol dipropionate). He confirmed the view that, although not strictly delimited, estrogen stimulates multiplication of ductal epithelium and that, when progesterone is combined, the effect is growth of acini and promotion of secretion. These changes are followed by fibrosis, and he too compared the condition to chronic cystic mastitis. The lesion may progress to sclerosing adenomatosis, a condition which Foote and

Stewart<sup>33</sup> attributed to alterations in acini rather than in ducts; however, acini have not been positively identified in human gynecomastia. In 2 of Eisen's 140 rats, adenocarcinoma developed, but there were no metastases and transplantation was not attempted. Thus there is only microscopic evidence of cancer. The possible fallacies of histologic diagnosis of malignant disease are well known, and confirmation by successful transplantation is desirable. The work on estrogenic hormones has been reviewed by Gardner,<sup>99</sup> by Allen,<sup>76</sup> and by Shimkin,<sup>62</sup> who stressed the importance of certain features. Genetic factors cause variations in response in different species and strains. The carcinogenic action of estrogens is especially effective on organs which are naturally stimulated, and is generally proportional to their physiologic activity, *i.e.*, up to certain limits the action reflects the dosage. In mice, the milk factor is highly significant, but is not the sole determiner of the effects.

Observation justifies the conclusion that in men estrogenic substances provoke the development of gynecomastia, but there is no satisfactory evidence that the lesion becomes cancerous. In male mice and rats, natural and synthetic estrogens induce proliferative and cystic changes with occasional development of carcinoma. It cannot be said, however, that the noncancerous lesion in rodents is the equivalent of gynecomastia in man; rather it resembles chronic cystic mastitis of women.

The enlargement of the breast in gynecomastia is undoubtedly due in large part to the growth of connective tissue. Von Haam and Cappel<sup>100</sup> pointed out conflicting reports in the literature as to the effects of hormones on the multiplication and growth of fibroblasts. In their experiments they used the hearts of mouse embryos to obtain tissue cultures of fibroblasts, and applied theelin, progesterone, and testosterone in crystalline form. In low concentrations estrin produced a slight increase in mitotic activity and a moderate increase of surface area of growth, but higher concentrations were inhibitory. Progesterone and testosterone were about equally inhibitory. Although they concluded that these sex hormones "could not be regarded as significant growth-stimulating agents," the possibility still remains that in low concentration and acting over a considerable period of time, estrogenic substances might be responsible for the connective tissue proliferation in gynecomastia. Although perhaps not directly applicable, the fibro-matogenic effects of estrogen and its metahormones are suggestive.<sup>101</sup>

*Estrogens and Unilateral Gynecomastia.* I have found no reference to hormone assays in unilateral gynecomastia. Can it be said that the lesion in unilateral gynecomastia differs fundamentally from that of

bilateral involvement or that with which hormonal changes are associated? The lesion in one breast is identical to that in the other when the condition is bilateral. The microscopic appearance in gynecomastia of testicular choriocarcinoma is seen in Figure 53, in that of extra-genital choriocarcinoma in Figure 54, and the lesion resulting from treatment with stilbestrol in Figure 55. In these the epithelial proliferation is greater than in most unilateral lesions, but not in all, and the loose periductal tissue with inflammation and fibrosis of the stroma is essentially the same. Since the microscopic changes of unilateral gynecomastia cannot be distinguished from those of the bilateral type known to be associated with evident endocrine disorders, probably due to the influence of estrogens, it may be that estrogens also play a part in the pathogenesis of unilateral gynecomastia.

*Theories on Unilateral Gynecomastia.* All theories on unilateral gynecomastia are hypothetical and none is founded on hormonal assays. Although expressing doubt as to validity, Weber<sup>4</sup> cited Lacaille as having described 2 cases of unilateral acromegaly. Weber thought of unilateral gynecomastia as the result of "potential hemihypertrophy . . . ready to be converted into actual unilateral hypertrophy or gigantism by hormonal stimulation." Later he and Atkinson<sup>102</sup> phrased his views somewhat differently, stating that in certain persons a portion of one side of the body forms a "local soil" differing from that of the other side, so that the two sides vary in their reaction toward a general stimulus, *e.g.*, endocrine disorder. Recently Weber<sup>103</sup> repeated his view to the effect that "just as one half of the body is occasionally bigger than its fellow, it is probable that one half may be *potentially bigger*, in the sense that certain organs in that half may respond more readily to hormonal or other stimulation than organs in the other half."

Richardson<sup>50</sup> emphasized the action of estrogenic hormones. Levi<sup>104</sup> objected vigorously to Richardson's interpretation, questioning the unilateral activity of hormones. Medvei<sup>105</sup> raised doubts as to the views of both writers and introduced the factor of constitution. He quoted Bauer as stating that there are two forms of gynecomastia, a constitutional form "due directly to a chromosomal imbalance" and a hormonal form in which "hereditary factors, when present, operate through the medium of the endocrine system." Enough has been said above to discredit these unsupported hypotheses.

Exophthalmos of hyperthyroidism may be unilateral and Pochin<sup>106</sup> has called attention to the fact that retraction of the upper lid, Dalrymple's sign, may also be unilateral. Fuller Albright, in a personal statement, explained unilateral gynecomastia as due to a dif-

ference in receptors for endocrine stimuli. Pochin, also in a personal communication, elaborated the views of Weber<sup>103</sup> and of Albright to the effect that the difference in receptors is not obvious when the endocrine stimulus is massive, but that slight stimuli may meet receptors somewhat more sensitive on one side than the other.

Pseudogynecomastia is only rarely unilateral. It is not clear that there is hormonal basis other than that occasionally operative in obesity.<sup>7</sup> Endocrine substances might be increased to such a slight degree that assays would not be delicate enough to reveal the change from normal, although by operating over long periods on sensitive receptors the stimulus might be effective.

### TREATMENT

If treatment is required, it should be surgical. Deaver and McFarland<sup>11</sup> gave the indications for surgical treatment as anxiety, mortification, or pain. To these must be added environmental conditions which subject the breast to friction or injury and also the suspicion that the lesion is neoplastic.

Endocrine products are ineffectual in the treatment of gynecomastia. Young<sup>107</sup> treated 6 patients with "pituitary lactogenic substance," testosterone, and progesterone, without observable effect. Sullivan and Munslow<sup>22</sup> used methyl testosterone in 1 case unsuccessfully. Dunn,<sup>13</sup> cited McCullagh and McGurl as having been successful in the local injections of male sex hormone but pointed out that there has been no confirmation over the course of 4 years and said that his own results were negative. Klinefelter, Reifenstein, and Albright<sup>63</sup> found that no favorable effects resulted from administration of estradiol dipropionate, progesterone, pregneninolone, or testosterone propionate.

Generally speaking, there is no reason for biopsy. The removal of the breast is a simple operation and a cross section of the lesion usually suffices to distinguish between gynecomastia and neoplasm, although microscopic examination gives the final decision. Depending on circumstances, the operation may be designed to remove mammary gland, areola, nipple, and skin, leaving enough skin to permit immediate closure, or it may be a subcutaneous resection of the mammary gland, leaving areola and nipple, as described by Webster.<sup>16</sup>

### SUMMARY

#### *Definition*

Gynecomastia is an enlargement of the mammary gland or glands of males due to proliferation of connective tissue, dense in the general stroma and often loosely arranged in periductal regions, together with

variable degrees of multiplication, elongation, or branching of ducts, or of all three, without formation of true acini, accompanied by periductal or more widespread infiltration of lymphocytes, plasma cells, large mononuclear cells, and occasionally eosinophils or neutrophilic polymorphonuclear cells, or both; secretion is frequently present in ducts, may be discharged spontaneously or manually expressed, but rarely if ever is it true colostrum or milk.

This definition excludes pseudogynecomastia, due to the deposition of fat in the mammary region, as well as suppurative or other essentially inflammatory processes, granulomatous lesions, and neoplasms, either benign or malignant.

### *Etiology*

Gynecomastia is much more frequently unilateral than bilateral, and the unilateral lesions are about equally divided between the two sides. It may occur at almost any time of life, but is especially frequent in the third decade. Only rarely does it succeed directly upon the pubescent subareolar node. After reaching a certain size, which varies widely from case to case, growth ceases, but reduction in size is rare. Resolution, to the point of complete cicatrization, occurs in breasts with active proliferation, but is limited to small foci and does not indicate resolution of the whole lesion. There is no clear indication that true neoplasia supervenes.

When gynecomastia accompanies manifest endocrine disorders it is usually bilateral, but not always equal, and occasionally only one breast is enlarged. In such cases, epithelial proliferation is conspicuous, but it is no greater than that frequently seen in unilateral gynecomastia without demonstrated endocrine disturbance.

From present knowledge of assays of hormones, no generalizations can be made. In obvious endocrine disorders, hormonal imbalance is observed, but increase in estrogenic substances is frequent. Experimental results and observations on man, when correlated, point toward estrin as the important factor. Even though androgens may excite proliferation in the mammary gland of experimental animals, there is little if any evidence that they, of themselves, have the same effect in man. Unilateral enlargement may possibly be due to hormonal imbalance or hyperestrinism acting over a long time on receptors especially sensitive on the one side, but of such small proportions as not to be demonstrable by current methods of assay.

Trauma or irritation may draw attention to the lesion and may perhaps augment growth, but there is no satisfactory evidence that either one is causative, nor is there evidence that hereditary or constitutional

factors (other than those implied in certain endocrine disorders) play any significant part.

When treatment is required, it should be surgical. Results have not justified the use of endocrine products in treatment of gynecomastia.

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## DESCRIPTION OF PLATES

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### PLATE 54

- FIG. 1. Bilateral gynecomastia in a young man with entirely male characteristics.
- FIG. 2. Unilateral gynecomastia in a young Negro of marked masculinity.
- FIG. 3. Elongation, distention, and branching of ducts.  $\times 10$ . Acc. no. 92355.

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3



Karsner

Gynecomastia

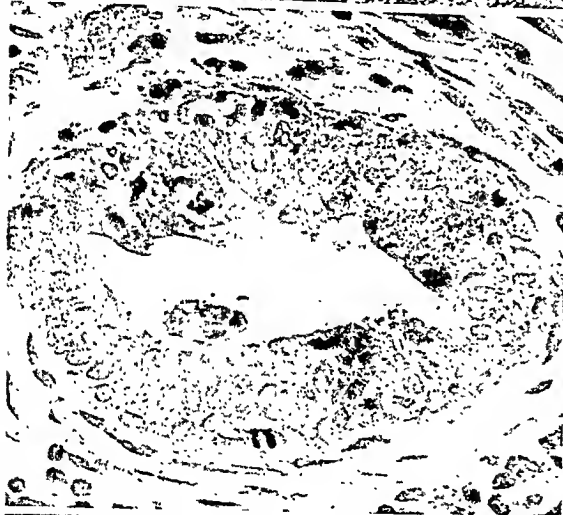
PLATE 55

- FIG. 4. Marked increase in number of ducts.  $\times 10$ . Army Institute of Pathology Acc. no. 85377.
- FIG. 5. Mitotic figure in basal layer of the epithelium of a duct.  $\times 354$ . Acc. no. 67499.
- FIG. 6. Mitotic figure in middle layer of the epithelium of a duct.  $\times 624$ . Acc. no. 98307.
- FIG. 7. Mitotic figure in luminal layer of the epithelium of a duct.  $\times 354$ . Acc. no. 94770.
- FIG. 8. Multiplication of epithelium of duct with formation of epithelial sprouts projecting into the lumen. There are fibroblasts and mononuclear cells in the periductal tissue.  $\times 142$ . Acc. no. 71373.

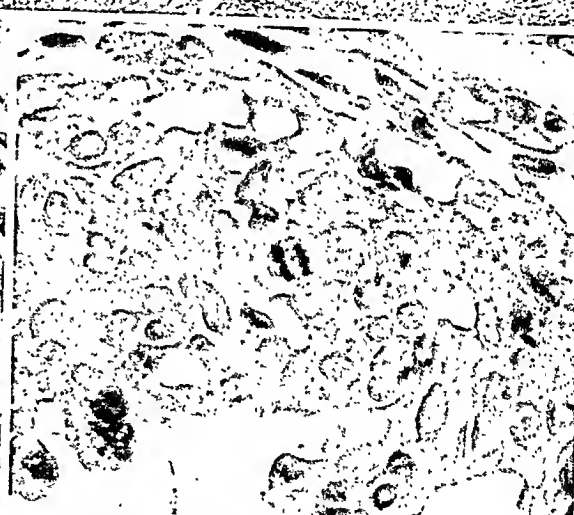
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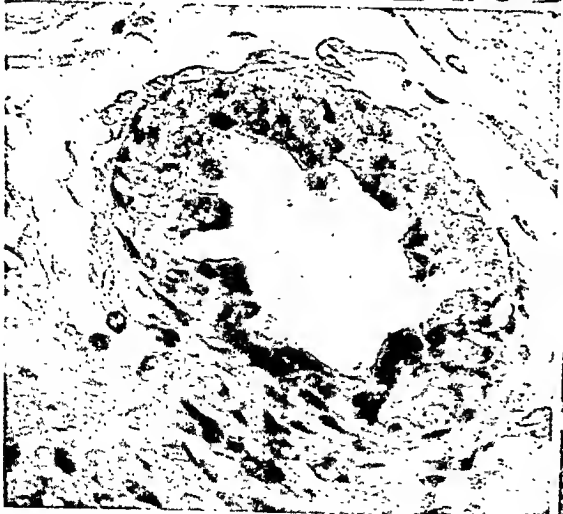
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Karsner

Gynecomastia



PLATE 56

FIG. 9. Elongation of epithelial cells toward cylindrical form, with intraductal sprouts.  $\times 354$ . Acc. no. 67449.

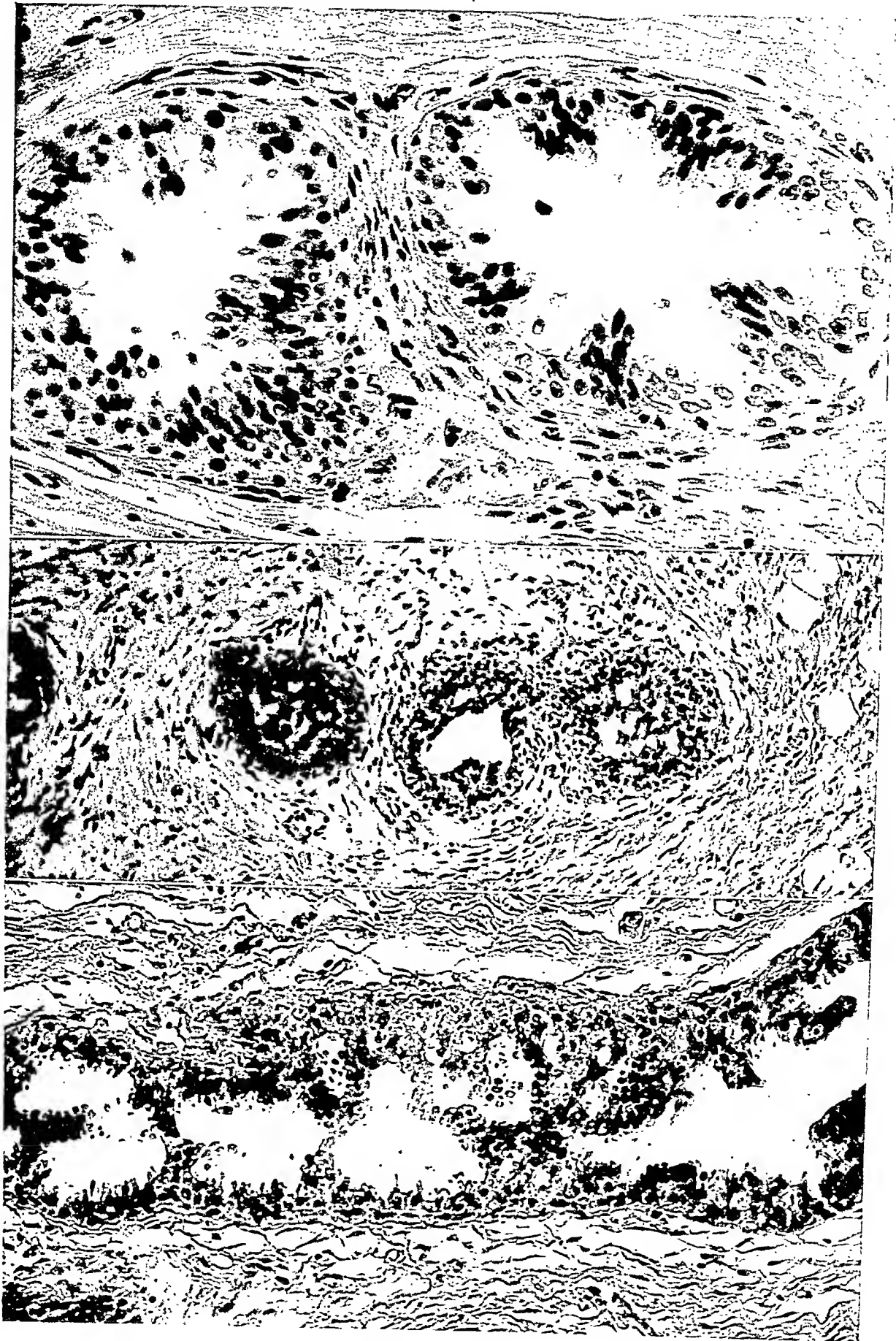
FIG. 10. Multiplication of epithelium of duct and fusion of sprouts to form locules, viewed in cross section.  $\times 142$ . Acc. no. 78890.

FIG. 11. Multiplication of epithelium of duct and fusion of sprouts to form locules, viewed in longitudinal section.  $\times 215$ . Acc. no. 99887.

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Karsner

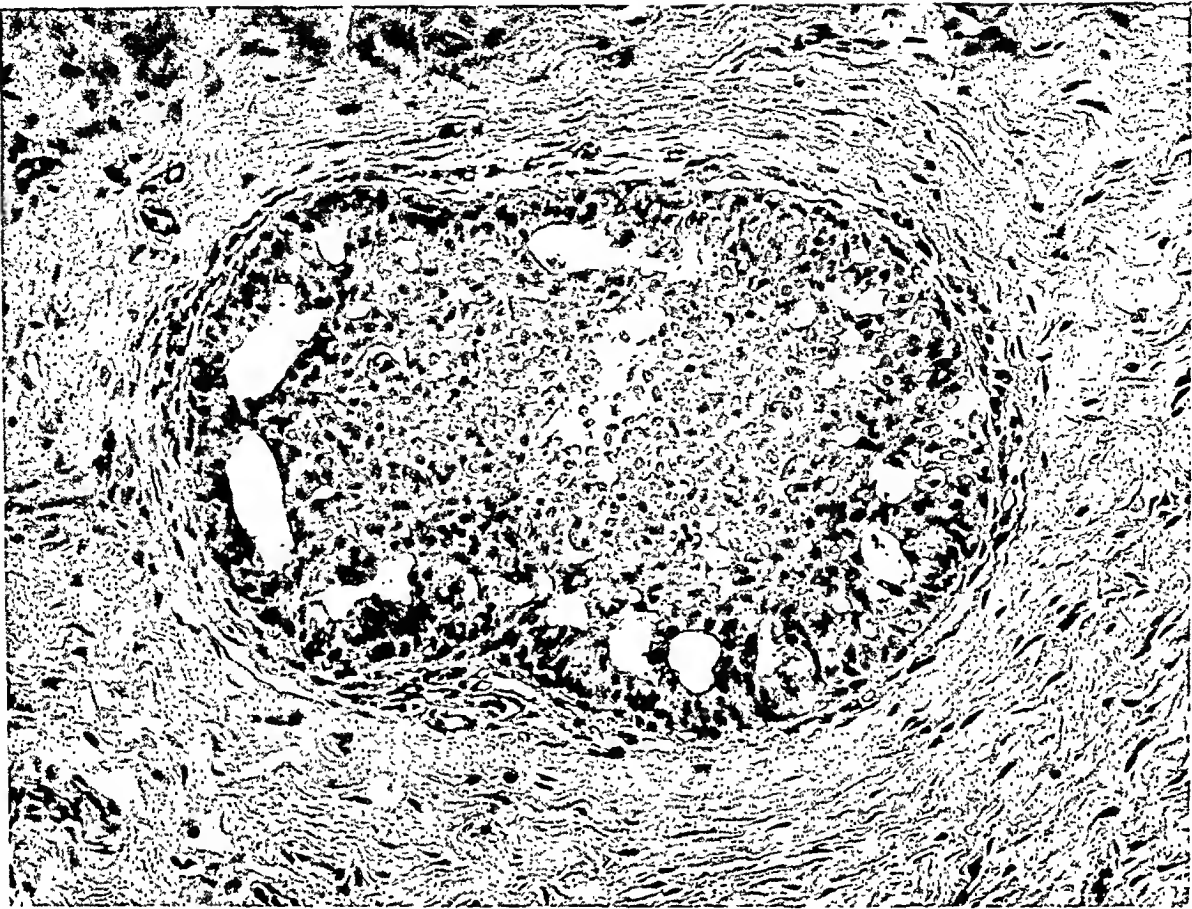
Gynecomastia

PLATE 57

FIG. 12. Multiplication of epithelium of duct, filling the lumen.  $\times 215$ . Acc. no. 78890.

FIG. 13. Elongation and branching of a duct with budding at the ends of two branches.  $\times 102$ . Acc. no. 106546.

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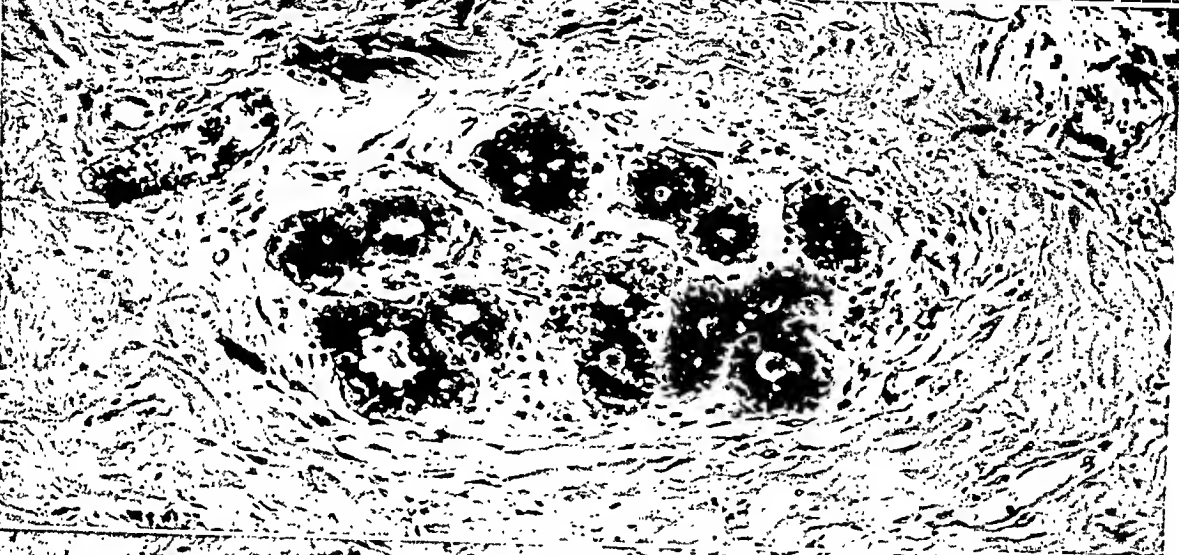
PLATE 58

- FIG. 14. Branching of duct with budding, as seen in cross section. There is a notable mononuclear cellular infiltration.  $\times 142$ . Acc. no. 78302.
- FIG. 15. Cross section of buds of a duct at a level not including the duct of origin, suggesting lobules but with multiple lining layers.  $\times 142$ . Acc. no. 94960.
- FIG. 16. Papillae with connective tissue cores in a slightly distended duct.  $\times 150$ . Acc. no. 86111.

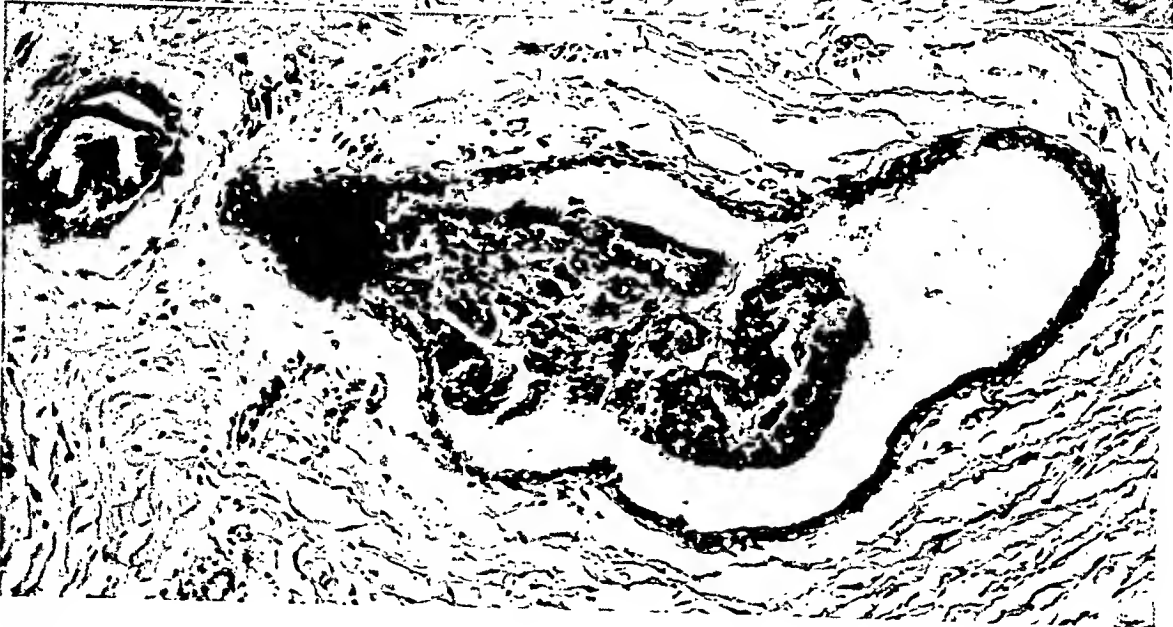
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Karsner

Gynecomastia

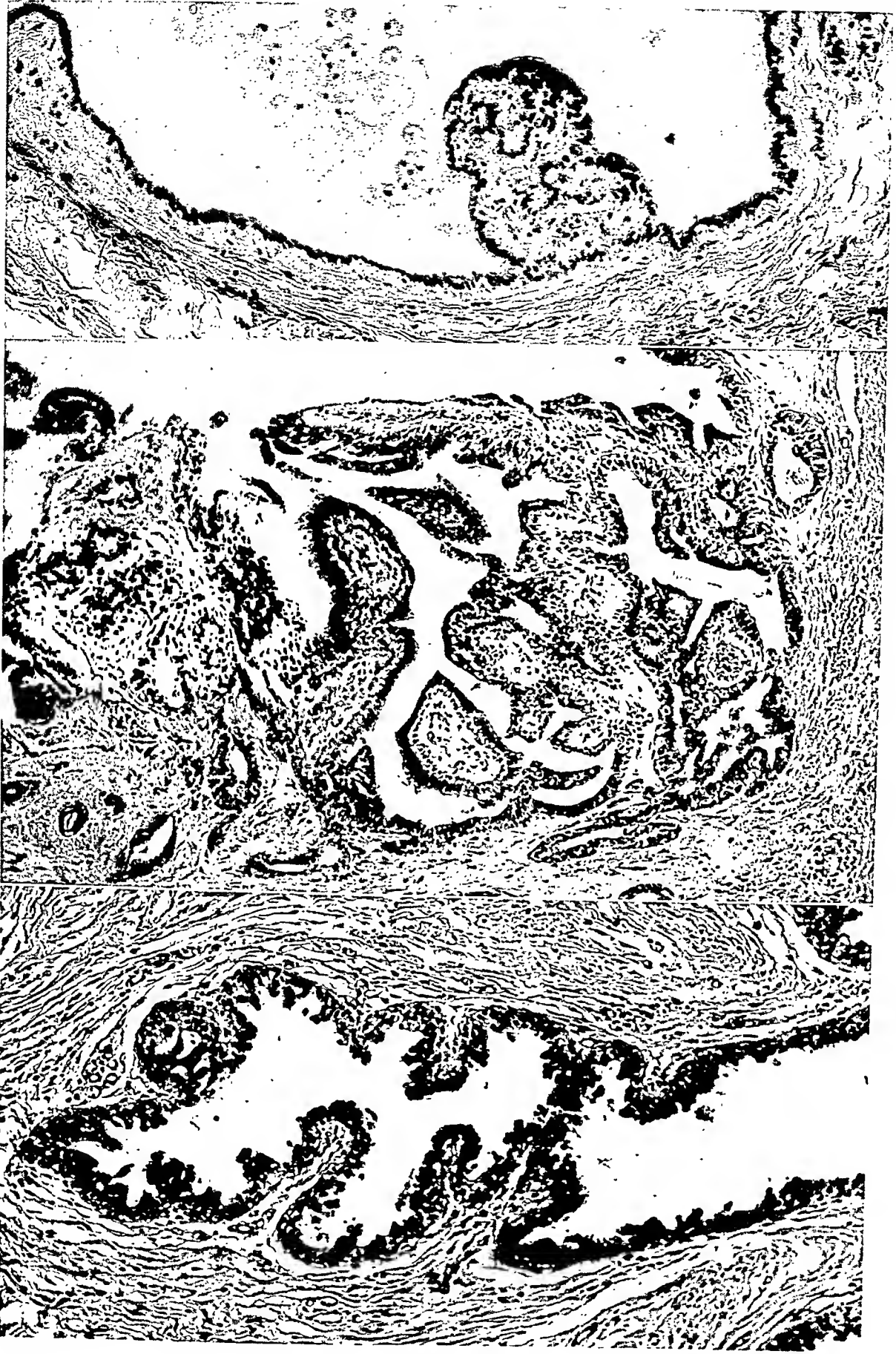
PLATE 59

FIG. 17. Papilla with connective tissue core in a markedly distended duct which contains acidophilic precipitate and desquamated epithelial cells.  $\times 142$ . Acc. no. 99858.

FIG. 18. Branching papillae with connective tissue cores in a distended duct.  $\times 109$ . Acc. no. 94551.

FIG. 19. Deformity of outline of duct with connective tissue growth projecting into lumen.  $\times 142$ . Acc. no. 72255.





Karsner

Gynecomastia



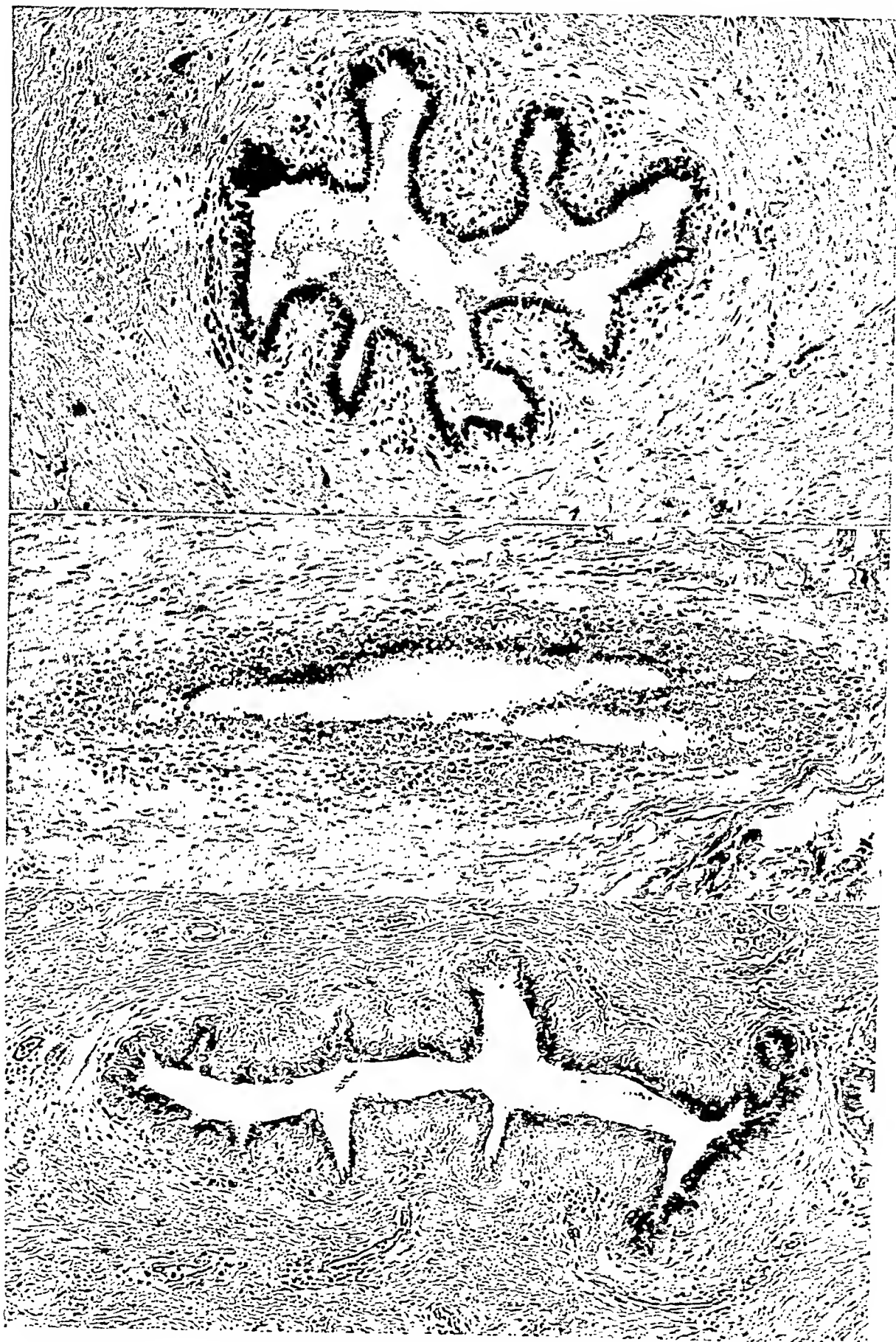
PLATE 60

- FIG. 20. Deformity of outline of duct with connective tissue growth projecting into lumen. Granular acidophilic precipitate in lumen and periductal infiltration of mononuclear cells.  $\times 142$ . Acc. no. 70626.
- FIG. 21. Duct of nipple with apparent deformity due to line of section. Periductal infiltration with mononuclear cells.  $\times 124$ . Acc. no. 82300.
- FIG. 22. Deformity of duct with connective tissue growth forming large, blunt projections into lumen. No whorling of connective tissue in the projecting masses.  $\times 109$ . Acc. no. 93620.

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Karsner

Gynecomastia

PLATE 61

FIG. 23. Acidophilic, finely granular precipitate in lumina of ducts, the epithelium of which has multiplied.  $\times 354$ . Acc. no. 76253.

FIG. 24. Duct of nipple containing finely granular, basophilic precipitate and desquamated cells. Suggests colostrum but is devoid of fat.  $\times 110$ . Acc. no. 84727.

FIG. 25. Mucinous cylindrical epithelial cells.  $\times 280$ . Acc. no. 93817.

FIG. 26. Squamous cell metaplasia in a slightly distended duct.  $\times 142$ . Acc. no. 60405.

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Karsner

Gynecomastia

PLATE 62

- FIG. 27. Ducts lined by multiple layers of large epithelial cells with acidophilic cytoplasm.  $\times 142$ . Acc. no. 67499.
- FIG. 28. Duct with conglutinated erythrocytes in lumen.  $\times 179$ . Acc. no. 60022.
- FIG. 29. Duct with lymphocytes in lumen.  $\times 179$ . Acc. no. 75838.
- FIG. 30. Infiltration of lymphocytes, plasma cells, and large mononuclear cells in loose periductal tissue.  $\times 354$ . Acc. no. 98568.

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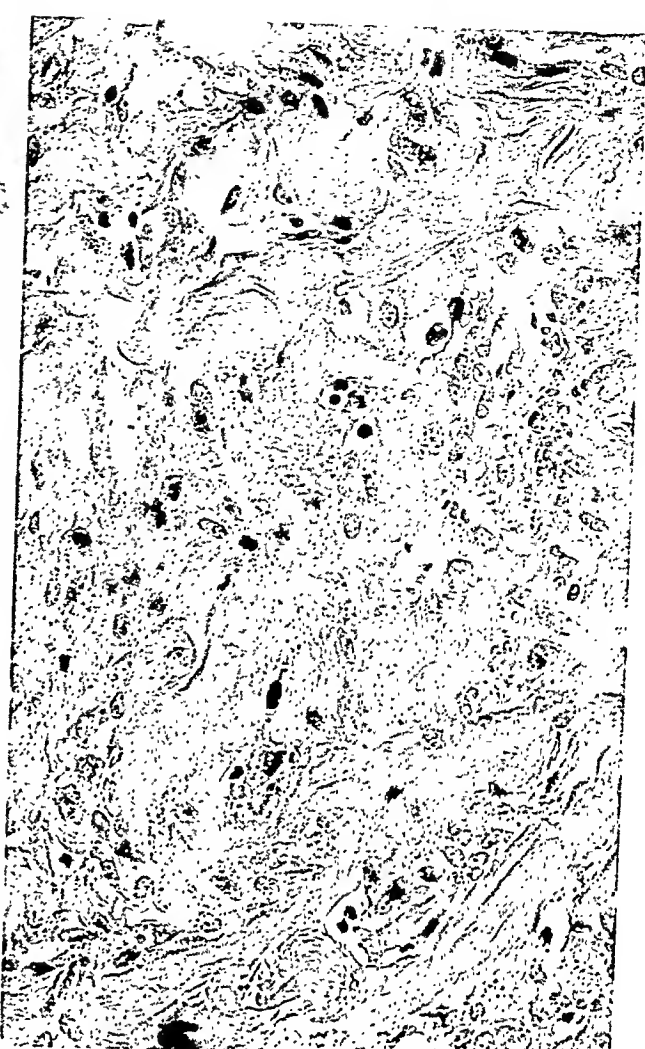
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Karsner

Gynecomastia

PLATE 63

- FIG. 31. Infiltration of lymphocytes, plasma cells, and large mononuclear cells in loose periductal tissue.  $\times 900$ . Acc. no. 78549.
- FIG. 32. Perivascular infiltration of mononuclear cells in stroma of mammary gland.  $\times 354$ . Acc. no. 97872.
- FIG. 33. Numerous fibroblasts in dense stroma of mammary gland.  $\times 252$ . Acc. no. 65257.
- FIG. 34. Proliferating capillaries in moderately loose periductal tissue.  $\times 142$ . Acc. no. 76999.



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34

Karsner

Gynecomastia



PLATE 64

- FIG. 35. Capillaries, fibroblasts, and mononuclear cells in loose periductal connective tissue.  $\times 142$ . Acc. no. 96406.
- FIG. 36. Exudate around and within a duct, with desquamation of lining epithelium.  $\times 142$ . Acc. no. 84978.
- FIG. 37. Massive exudation around a duct with slight exudation within the duct. Partial denudation of epithelial lining.  $\times 215$ . Acc. no. 86302.

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Karsner

Gynecomastia

PLATE 65

- FIG. 38. Localized proliferation of ducts, not encapsulated.  $\times 10$ . Acc. no. 83595.
- FIG. 39. Detail of Figure 38, to show ductal proliferation, epithelial multiplication, and infiltration of mononuclear cells into loose periductal tissue.  $\times 142$ . Acc. no. 83595.
- FIG. 40. Elongation of intraductal epithelium, suggestive of intraductal carcinoma, but without pleomorphism or invasion.  $\times 179$ . Acc. no. 73774.

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Karsner

Gynecomastia

PLATE 66

FIG. 41. Desquamation of lining epithelium of duct, with partial denudation of wall and basophilic, finely granular precipitate.  $\times 117$ . Acc. no. 86095.

FIG. 42. Partial denudation of lining epithelium. Many distended capillaries in periductal connective tissue.  $\times 215$ . Acc. no. 92335.

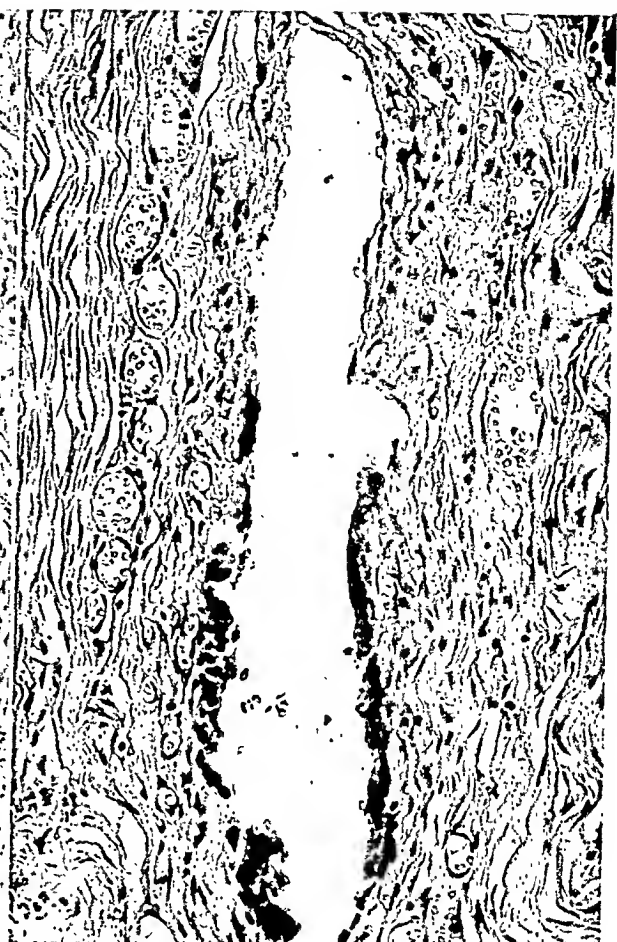
FIG. 43. Partial denudation of lining epithelium of duct with surrounding inflammation and condensed mass of epithelium at one side.  $\times 142$ . Acc. no. 86095.

FIG. 44. Condensation of ductal epithelium within a duct surrounded by granulation tissue.  $\times 179$ . Acc. no. 67605.

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Karsner

Gynecomastia

PLATE 67

- FIG. 45. Almost complete disappearance of ductal epithelium. Mass of granulation tissue surrounding the remains of the lumen.  $\times 252$ . Acc. no. 99820.
- FIG. 46. Disappearance of lining epithelium and preservation of hyaline basement membrane of a duct, with a surrounding mass of granulation tissue.  $\times 179$ . Acc. no. 67499.
- FIG. 47. Complete disappearance of ductal lumen, with a central mass of granulation tissue surrounded by fibroblastic tissue.  $\times 173$ . Acc. no. 88324.



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PLATE 68

FIG. 48. Tightly coiled sudoriferous sweat gland, not abnormal.  $\times 109$ . Acc. no. 77430.

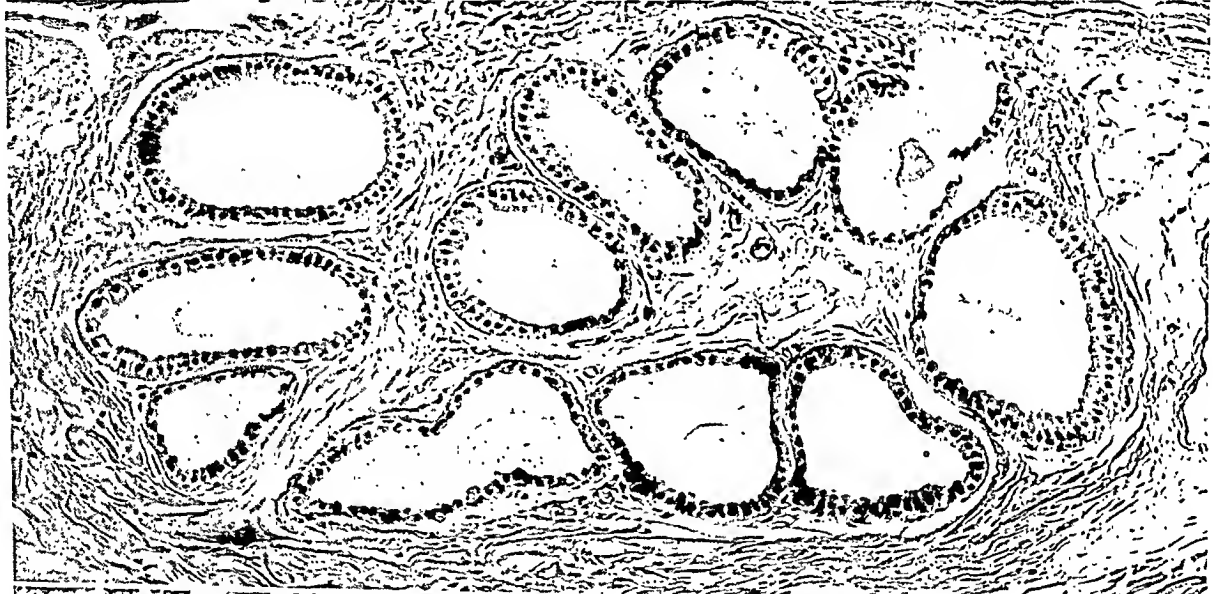
FIG. 49. Loosely coiled, relatively inactive, apocrine gland.  $\times 109$ . Acc. no. 81678.

FIG. 50. Apocrine gland in late stage of activity, with granular secretion in lumina.  $\times 109$ . Acc. no. 75250.

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Karsner

Gynecomastia

PLATE 69

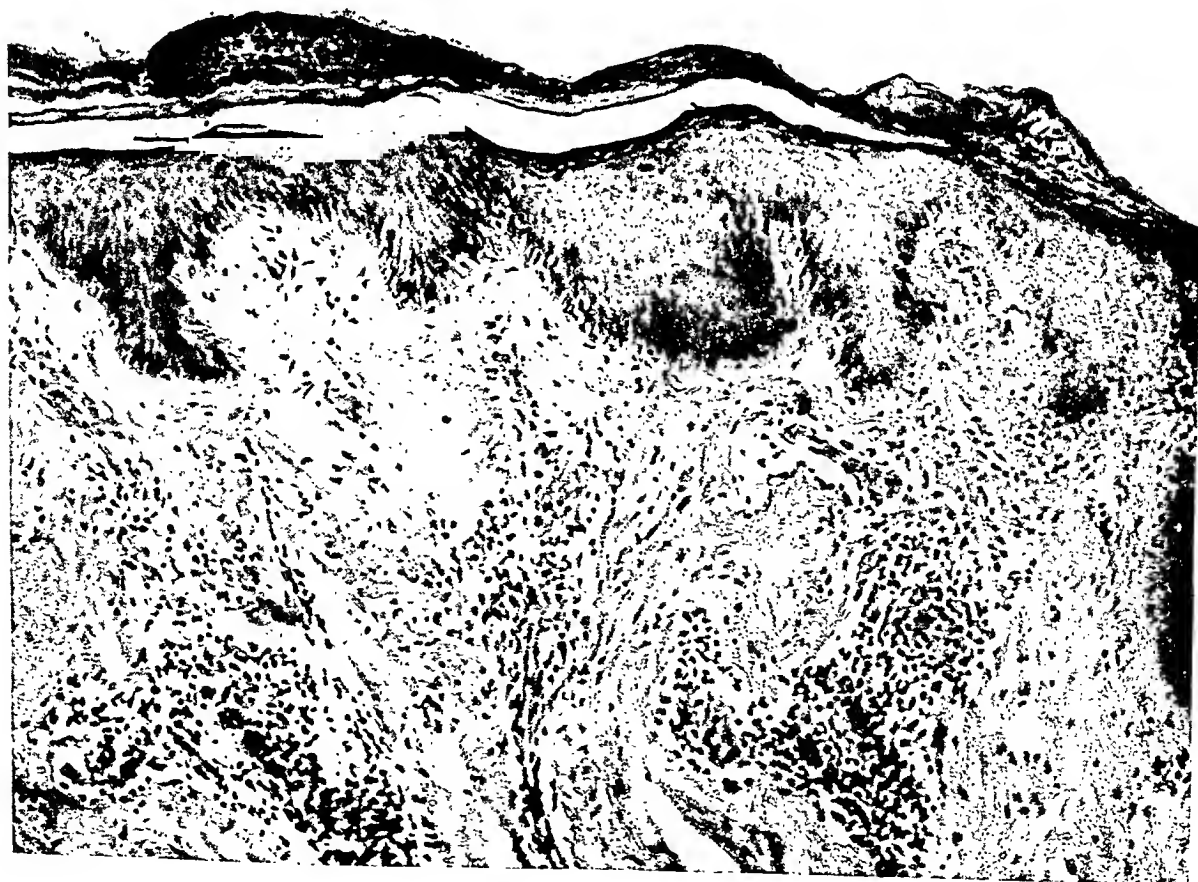
FIG. 51. Subacute dermatitis with dyskeratosis and also infiltration of mononuclear cells in papillary and upper reticular layers.  $\times 142$ . Acc. no. 89770.

FIG. 52. Subacute dermatitis with crust formation and infiltration of mononuclear cells in papillary and upper reticular layers.  $\times 142$ . Acc. no. 89770.

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Karsner

Gynecomastia

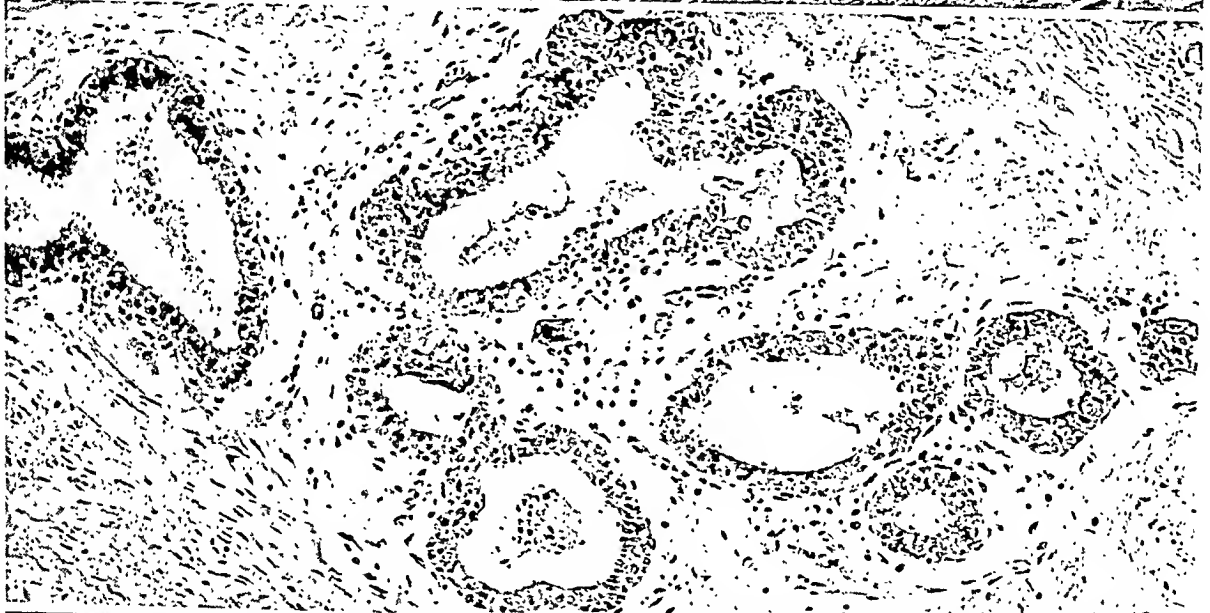
PLATE 70

- FIG. 53. Multiplication of lining epithelium, and infiltration of mononuclear cells in periductal tissues. Case of choriocarcinoma of testis.  $\times 142$ . Acc. no. 93056.
- FIG. 54. Multiplication of lining epithelium and infiltration of mononuclear cells in loose periductal tissue. Case of choriocarcinoma originating in thorax (Laipply and Shipley<sup>60</sup>).  $\times 142$ . Western Reserve University, Inst. Path., A 8014.
- FIG. 55. Multiplication of lining epithelium and infiltration of mononuclear cells in loose periductal tissue. Prolonged administration of diethylstilbestrol in a case of carcinoma of the prostate, a white man, 70 years old.  $\times 160$ . Acc. no. 75980.

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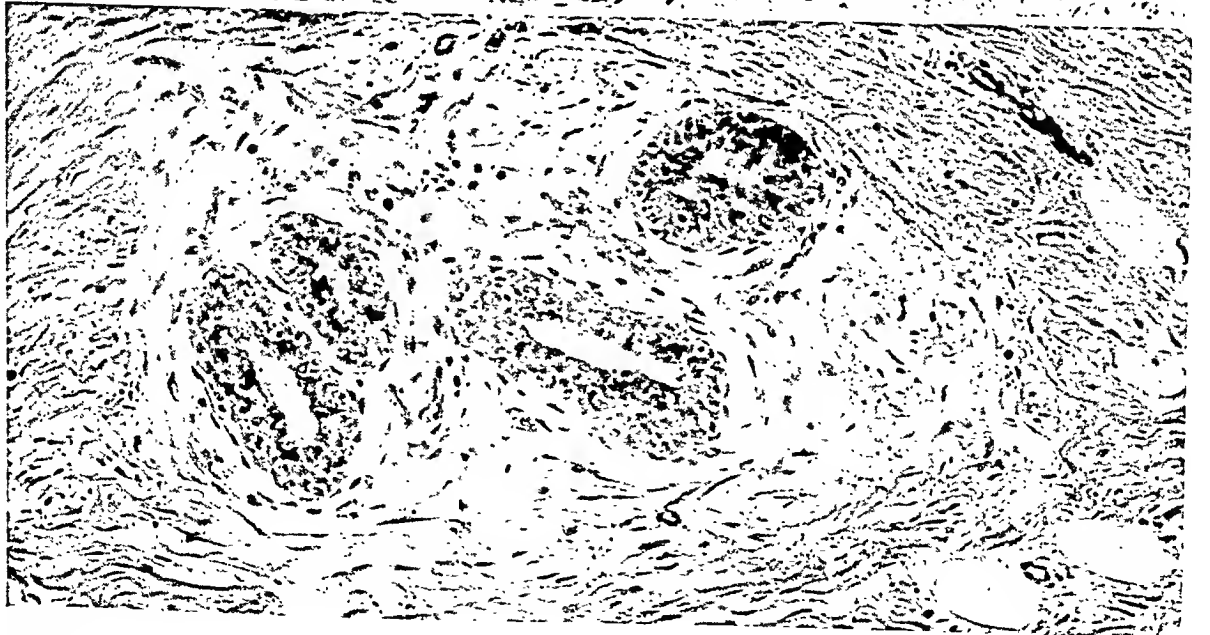


PLATE 71

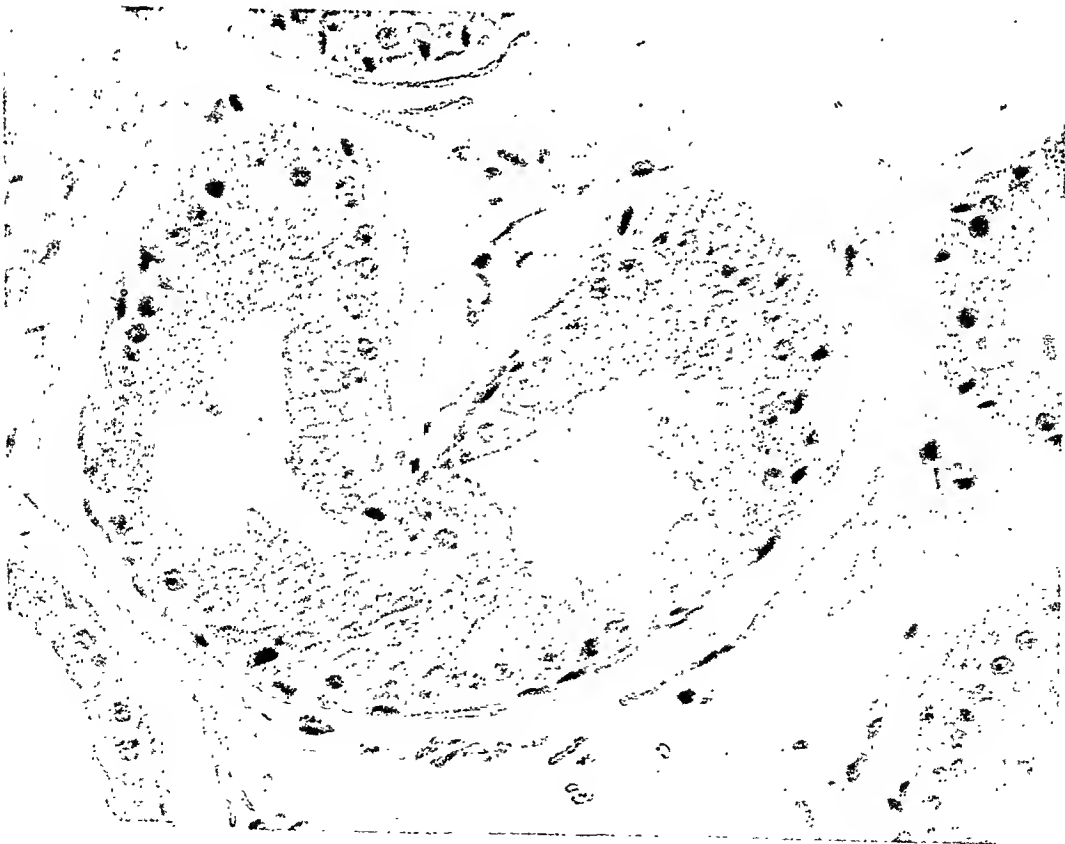
FIG. 56. Photomicrograph by Ansco color process to show mucoid degeneration in loosely arranged, inflamed, periductal connective tissue.  $\times 135$ . Acc. no. 97594.

FIG. 57. Photomicrograph by Ansco color process to show apocrine gland in secretory phase with acidophilic globules in cytoplasm. Of note is the myoepithelium.  $\times 350$ . Acc. no. 77829.

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Karsner

Gynecomastia





# STUDIES ON THE EARLY CHANGES IN THE LIVERS OF RATS TREATED WITH VARIOUS TOXIC AGENTS, WITH ESPECIAL REFERENCE TO THE VASCULAR LESIONS

## II. THE HISTOLOGY OF THE RAT'S LIVER IN ALLYL FORMATE POISONING \*

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The present report is one of a series dealing with the initial effect of various toxic agents on the vascular system and the parenchyma of the liver.<sup>1</sup> It is concerned with the early changes occurring in allyl formate (2-propenyl methanoate,  $\text{HCOO-CH}_2\text{-CHCH}_2$ ) intoxication.

### MATERIAL AND METHODS

Observations were made on 34 albino rats whose average weight was about 100 gm. Allyl formate was administered by the intraperitoneal route. Thirty rats received a single injection of 0.015 cc. per 100 gm. of body weight of allyl formate diluted in 2 cc. of Ringer's solution; they died or were sacrificed 1 to 2, 3 to 6, and 24 hours after administration of the drug. A group of 4 rats which survived 24 hours were given a second dose of 0.015 cc. of allyl formate per 100 gm. of body weight; all of these latter animals died 2 to 7 hours after receiving the second dose.

Pieces of liver were fixed in a 4 per cent solution of formaldehyde, in Carnoy's fluid, and in Zenker's solution, and were blocked in celloidin-paraffin. Sections, 6  $\mu$  in thickness, were stained with hematoxylin and eosin, Heidenhain's iron-hematoxylin, and with Mallory's anilin blue collagen stain. Frozen sections stained with sudan III were used for the study of fats.

### RESULTS

#### A. Findings in Rats Examined 1 to 2 Hours after Administration of Allyl Formate

Macroscopically, the livers appeared enlarged and dark red<sup>‡</sup> 1 to 2 hours after administration of allyl formate. On microscopic examination the most striking features were the strong capillary congestion and the damage of the wall of the sinusoidal capillaries (Figs. 1-3). The

\*Received for publication, March 20, 1945.

† Working under a grant in aid given by Mrs. J. H. Stodel, Cape Town, South Africa.

‡ The gross changes of the livers were the same in all series of experiments with a single dose of allyl formate. For this reason no further mention will be made of the gross appearance of the organ.

congestion of the sinusoids was most marked in the peripheral third of the lobule, though occasionally it extended throughout the whole lobule. The sinusoids were greatly widened and engorged with red blood cells; the liver cell cords often appeared compressed to small thread-like bands. The capillary wall was usually swollen, hyaline, and often disrupted. The Kupffer cells were frequently detached; their cytoplasm was sometimes strongly eosinophilic and the nuclei pyknotic. In many places the distended sinusoidal clefts were entirely devoid of endothelial lining and the fragments of the capillary wall were found among the red blood cells between the liver cell cords. In other places, where no rupture of the capillary wall occurred, the walls of the sinusoidal capillaries were in close contact with the liver cells, the pericapillary spaces being only rarely or not at all visible. There were no signs of pericapillary edema.

The vascular damages were not limited to the sinusoidal capillaries but also affected the portal and sublobular veins. Their endothelium was often swollen or desquamated and their wall hyaline and structureless. The connective tissue around the portal canals was edematous and the accompanying lymphatics were greatly distended (Fig. 4). Occasionally the portal and sublobular veins were surrounded by areas containing extravasated erythrocytes (Fig. 5) and eosinophilic granular material, resembling precipitated albumin. In the walls of the portal veins the fibers were often loosened and there were erythrocytes between them.

At this stage the epithelial parenchyma of the liver showed no appreciable alterations, though a certain number of liver cells situated in the zones of intensive capillary damage displayed the signs of early hydropic degeneration. They were somewhat swollen and their cytoplasm contained either several well defined smaller vacuoles or one or two larger ones (Fig. 6). These vacuoles usually appeared to be empty. In some cases, however, they contained a homogeneous transparent material which stained faintly pink with eosin and occasionally one or several erythrocytes could be observed within these vacuoles. There was no fat in the liver cells. The nuclei of the liver cells were normal in size and shape, with well defined nuclear membrane.

#### *B. Findings in Rats Examined 3 to 6 Hours after Administration of Allyl Formate*

Three to six hours after administration of allyl formate the damages in the portal and sublobular veins were more accentuated. Many of them were now surrounded by rather broad hemorrhagic areas. The sinusoidal capillaries, especially those in the peripheral parts of the

lobule, were destroyed, and fragments of the capillary lining were often found in the distended sinusoidal clefts together with pinkish staining granular material, detached Kupffer cells, and erythrocytes. The rupture of the capillaries resulted in the formation of small blood pools.

The hydropic vacuolization of the liver cells may reach an extensive degree at this stage, and in many cases it affected the whole of the epithelial parenchyma. The presence of fat could not be demonstrated in the vacuoles; many of them contained homogeneous material which stained uniformly pink with eosin, and also red blood corpuscles, single or in small groups.

In rat 17 the pathologic picture was especially illustrative. In this animal the ingestion of erythrocytes by the liver cells had attained an extreme degree. The trabecular tracery of the liver lobules was greatly obscured in the peripheral areas, which often presented a completely uniform, sieve-like appearance (Fig. 7). Examination at higher magnification showed that most of the liver cells in these areas were swollen and possessed a large vacuole. The vacuoles were sometimes empty, but frequently contained red blood cells as well as a hyaline material, which stained slightly pink with eosin (Figs. 8-13). The numbers of ingested erythrocytes varied from a few to a great many. When they were numerous, they filled the vacuoles completely and were massed together to form larger conglomerates. The individual corpuscles within the conglomerates were well defined and well stained. When the number of ingested erythrocytes was small, the liver cells retained their characteristic shape and size. As the inclusions increased in volume the nuclei were pushed aside and the protoplasmic rim became narrower. Finally the cytoplasm was reduced to a very thin ring staining reddish brown with hematoxylin and eosin, and the flattened nuclei appeared as sickle-shaped bodies. The distended hepatic cells were pressed so closely one to another that the intercellular spaces between them appeared either very small or completely obliterated.

### *C. Findings in Rats Examined 24 Hours after Administration of Allyl Formate*

The portal and sublobular veins of rats examined 24 hours after administration of allyl formate often exhibited severe changes, amounting occasionally to complete destruction of the vessel walls. But also at this stage the vessels were not uniformly damaged, and side by side with markedly affected veins numerous others were encountered appearing to be intact. Perivascular edema was not present, although the perivascular hemorrhagic areas were often extensive. The changes in capillaries were similar to those described in the foregoing paragraph.

At this stage the liver cells in the peripheral areas of the lobule showed the first definite signs of necrosis. They were swollen and their homogeneous cytoplasm stained reddish brown with eosin. Many of the nuclei became poorly defined or invisible, others were irregularly shrunken and fragmented. The outlines of the cells could often be distinguished only with difficulty. The necrotic areas were sharply limited. The hepatic cells in the central parts of the lobule were always well preserved.

*D. Findings in Rats Which Received Two Injections of Allyl Formate*

Rats surviving 24 hours were injected with a second dose of 0.01 cc. of allyl formate. They died 2 to 7 hours after administration of the second dose.

The livers were swollen, congested, brownish red, and showed an interlacing network of red lines.

The most striking feature of the liver changes in rats of this series was the extensive hemorrhages associated with widespread parenchymal necrosis. The portal and sublobular veins were constantly surrounded by broad hemorrhagic zones of irregular outline which often extended as wide tracts from one portal area to the next (Fig. 14). The walls of the veins situated in the hemorrhagic areas were greatly thickened and had a hyaline appearance. They were often devoid of endothelial lining and contained erythrocytes between the collagenous fibers. Sometimes the veins were completely destroyed and their former position could only be recognized by the accompanying arteries and bile ducts.

The perivascular hemorrhages pushed aside the liver tissue surrounding the affected vessels, thus completely destroying the normal architecture of the liver. There were often no remnants of hepatic tissue left in the hemorrhagic areas. In other cases only the parenchymal cells disappeared in the affected zones; whereas the mesenchymal supporting stroma of the organ remained. Red blood corpuscles, detached Kupffer cells, and isolated liver cells were mingled with the reticular framework.

In places where the hemorrhages did not attain this extreme degree and did not replace the parenchyma around the periportal tracts, massive and widespread necrosis of the peripheral liver cells could be observed. It developed around the portal vessels, extended to the mid-zonal regions, and fused with similar necrotic areas of other lobules (Fig. 15). The cytoplasm of the liver cells appeared granular and

stained brownish red with eosin, probably because of intense imbibition with hemoglobin. The liver cells became swollen and their boundaries disappeared progressively. At first the nuclei persisted as structureless bluish brown masses; later they became dimly visible or were absent. Ingested red blood cells or their remnants could often be distinguished in the necrotic liver cells. Sometimes the liver cells showed a more advanced degree of necrosis with breaking up of cell bodies and complete obliteration of the intercellular spaces. Most of the central areas of the lobules showed no obvious departure from normal and persisted as islands among the necrotic tissue or hemorrhage; in addition there was often a comparatively narrow, unaffected zone, sometimes of a breadth of only one or two liver cells, bordering the portal venules. The necrotic areas were sharply defined, there being no gradual transition from these to areas of healthy tissue; immediately adjacent to cells showing complete necrosis were cells hardly affected at all. The nuclei of remaining liver cells were often very large; binucleated cells were numerous; no mitotic figures were seen. There was a slight leukocytic infiltration.

#### DISCUSSION

Piazza<sup>2</sup> was the first to report on the effect of allyl formate on the liver. Working with mice, rabbits, cats, and dogs, he studied the changes produced in the livers after doses of 0.05 gm. of allyl formate per kg. of body weight administered by the subcutaneous route. He gave no detailed description of the histologic changes but mentioned the vacuolization and necrosis of the peripheral liver cells, as well as widespread hemorrhages in the necrotic areas.

Eppinger, Kaunitz, and Popper<sup>3</sup> and Popper<sup>4</sup> studied the effect of allyl formate, after subcutaneous and oral administration, on the livers of dogs. They noted considerable sinusoidal congestion and the detachment of the walls of sinusoidal capillaries from the liver cell cords; the distended pericapillary spaces were found to be filled with an eosinophilic granular material and sometimes contained erythrocytes. At a more advanced stage there was widespread necrosis of parenchymal cells, the changes leading ultimately to cirrhosis of the liver. Similar alterations were observed by Popper<sup>5</sup> in the livers of salamanders after injection of allyl formate under the skin of the back.

Eppinger and co-workers<sup>3</sup> considered allyl formate to be a specific capillary poison and attributed the liver necrosis occurring in intoxication with allyl formate to capillary injuries, which interfere with the normal exchange between the parenchymal cells and the blood. The fact that allyl formate increases the permeability of the blood vessels

was demonstrated by Roller and Schober.<sup>6</sup> They injected the salamander intravenously, first with uranin solution and then with allyl formate. After the allyl formate injection the uranin solution leaked through the vessel walls and could be demonstrated photographically as fluorescent bands accompanying the blood vessels. Raff and Abrahamczik<sup>7</sup> reported that the permeability of animal membranes (ox gut) is increased under the influence of allyl formate and allylamine.

The view advocated by Eppinger and his associates<sup>3</sup> that allyl formate acts primarily as a vascular poison was opposed by Heinemann,<sup>8</sup> working in Aschoff's laboratory. She injected rats and rabbits with single or repeated doses of allyl formate. In rats, she observed the congestion of sinusoids, but no damage of the capillaries, no pericapillary edema, and no necrosis of the liver cells. In rabbits she noted periportal necrosis with hemorrhages in the necrotic areas, but no major capillary damages, and no detachment of the capillary walls from the liver cell cords. Heinemann claimed that in allyl formate intoxication the liver cell injuries precede the vascular damage, and concluded that allyl formate is not a capillary but a liver cell poison.

The problem of the nature of the liver changes in allyl formate poisoning is of especial interest for the following reason: In 1934, Rössle<sup>9</sup> described as "serous hepatitis" an inflammatory process in the liver, which affects the capillaries, increases their permeability, and leads to leakage of plasma into the intercellular spaces. The original injury to the capillaries is followed by dissociation and occasional disappearance of the liver cells as well as by development of collagenous fibers within the extravasated plasma. Rössle regarded this condition as the first and obligatory stage of those morbid processes in the liver which finally lead to cirrhosis of the organ. "Serous inflammation" may also involve other organs, according to this author. This concept was later adopted by Eppinger and his associates.<sup>3</sup> They went much further, considering "serous inflammation" as the almost universal pathognomonic basis for the great variety of parenchymatous damages in most organs. As far as the liver is concerned, their assumption is based almost exclusively on the observations made in animals poisoned with allyl formate, since, according to them, the changes observed in allyl formate intoxication and in serous hepatitis are completely identical. They considered allyl formate intoxication as an experimentally produced "serous hepatitis." The concept of "serous inflammation" has been prominent in the German literature during the past decade, but has been accepted with some reserve in the United States (Keschner and Klemperer,<sup>10</sup> Karsner,<sup>11</sup> Lichtman,<sup>12</sup> Moon<sup>13</sup>).

In analyzing our results, it may be stated that allyl formate produces in the livers of rats a series of profound changes which involve both the parenchyma and the vascular system. The retrograde processes in the parenchyma of the organ are invariably preceded by vascular lesions.

Even at the earliest stages investigated (1 to 2 hours after injection) the damage of sinusoidal capillaries in the livers of rats treated with

allyl formate is very pronounced. These alterations are not limited to disturbance in the permeability of the capillary wall, as assumed by Eppinger and his associates,<sup>3</sup> but are of a more far-reaching character. The capillary damage produced by allyl formate consists in definite disorganization and often in complete destruction of the walls of the sinusoidal capillaries in the periphery of the lobule. Not the pericapillary edema, but the disappearance of the capillary lining and the hemorrhages into the parenchyma are the constant and characteristic features of the lesions here described. The vascular changes just described are not confined to the lining of the sinusoidal capillaries but are similarly marked in the branches of the portal and sublobular veins. The swelling and disorganization of the endothelium of the intima, hyalinization of the media, as well as mural and perivascular edema, can be regularly observed. These changes ultimately lead to the complete destruction and rupture of the vessel wall, causing extensive and widespread hemorrhages around the portal tracts. The surrounding parenchyma becomes flooded with extravasated blood.

The changes observed in the liver cells may be summarized as follows: The definite lesions of the liver parenchyma appear at a later stage than those of the vascular system. In experiments of 1 to 2 hours' duration, *i.e.*, at a stage when the destruction of the sinusoidal capillaries is already most pronounced, the liver cells show no evidence of injury, except for some slight signs of hydropic degeneration. The hydropic degeneration becomes very intense 3 to 6 hours after the administration of the drug. In addition to hemal fluid the liver cells at this stage are found to contain in their cytoplasm large numbers of red blood corpuscles. However, even in the areas of strongest hydropic degeneration there is no necrosis of liver cells at this time; the nuclei of the liver cells are well preserved throughout. Necrosis of parenchymal cells becomes apparent only 24 hours after injection of allyl formate. The cells affected are all situated in the peripheral parts of the lobules, whereas the central regions are always well preserved.

The results of the present investigation indicate quite clearly that allyl formate, a drug which possesses a pronounced capacity to provoke intense and widespread necrosis of the liver parenchyma and which, after repeated administrations, may produce cirrhosis of the liver,<sup>3,4</sup> is a powerful vascular poison. The sequence of hepatic changes in rats treated with allyl formate appears to indicate that allyl formate affects primarily the vascular system.

The fact that the capillary lesions precede the retrograde changes in the liver cells, the local coincidence of both vascular and paren-



chymal alterations, as well as the special character of the earlier parenchymal injuries (hydropic vacuolization, ingestion of erythrocytes), all point to a causal relationship between the vascular lesions and the liver cell changes. From the above account it would appear that the primary intense damage of the capillaries creates conditions which favor or provoke retrograde processes in the liver cells, and that these retrograde changes take place in an internal environment altered by the breakdown of the blood-tissue barrier.

It is to be noted that the liver changes caused by allyl formate bear much resemblance to the changes which we<sup>1</sup> found in rats treated with urethane, another substance with definite vessel-damaging properties. Both poisons attack the capillary walls and the walls of the large veins; both provoke the breakdown of the barrier between blood and parenchyma, an event resulting in hemorrhages into the parenchyma; the alterations of the liver cells, too, are of a similar nature in both types of poisoning.

The effects in allyl formate intoxication differ from those in urethane poisoning in that in the former the vascular damages clearly precede the alterations of the parenchymal cells, whereas in the latter the vascular and parenchymal damages coincide. Moreover, there is a distinct difference in the degree of the vascular lesions. They are much more pronounced in rats treated with allyl formate than in rats treated with urethane. In the case of urethane we are dealing in the first place with disturbances in the permeability of the capillary wall, while allyl formate produces changes amounting to complete breaking up of the capillary system. The moderate changes in the vascular system of the livers of animals treated with urethane are accompanied by little advanced liver cell changes; the far-reaching destruction of the blood vessels in allyl formate poisoning are attended with extensive necrotic processes in the liver parenchyma.

The comparison of events in allyl formate and urethane poisoning appears of interest, as it seems to indicate a quantitative relationship between the vascular and parenchymal lesions. When a milder vascular poison is applied, the parenchymal damages are slight; the administration of a strong vascular poison is followed by extensive parenchymal necrosis. This comparison would tend to show that the condition of the liver cells is essentially dependent on the condition of the vascular wall, and that the degree of the parenchymal damage is a function of the degree of vascular injury.

The observations made in these investigations may be added to those which strengthen the view that with some poisons alterations of the vascular wall are concerned in the genesis of necrosis of the liver cells.

This assumption does not, of course, exclude the possibility of a direct effect on the liver parenchyma of the substance administered. This possibility cannot be evaluated on the basis of the present study and requires further investigation.

### SUMMARY

Intraperitoneal administration of allyl formate causes, in the livers of white rats, severe and widespread damage of sinusoidal capillaries and portal veins, followed by extravasation of plasma and formed elements of the blood into the parenchyma. At a stage when injury of the sinusoidal capillaries is already very pronounced, there is still little evidence of change in the liver cells, except for signs of beginning hydropic degeneration. The hydropic degeneration of the hepatic cells progresses and some hours later reaches an extreme degree. The liver cells in the periphery of the lobule contain, at this stage, in addition to hemal fluid, erythrocytes in large numbers. Continued action of the poison causes extensive hemorrhage into the periportal areas, as well as necrobiosis of the liver cells in the peripheral and midlobular zones.

The results of the foregoing observations strengthen the view that the condition of the liver cells is essentially dependent upon the condition of the vascular wall. The data available are, however, not sufficient to exclude a direct action on the liver parenchyma of the substance administered.

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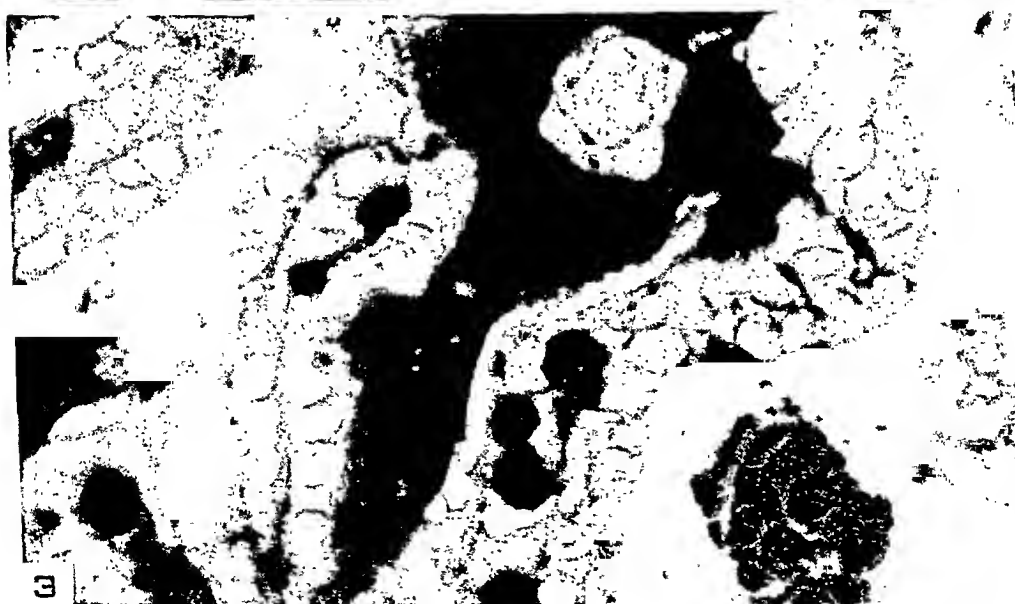
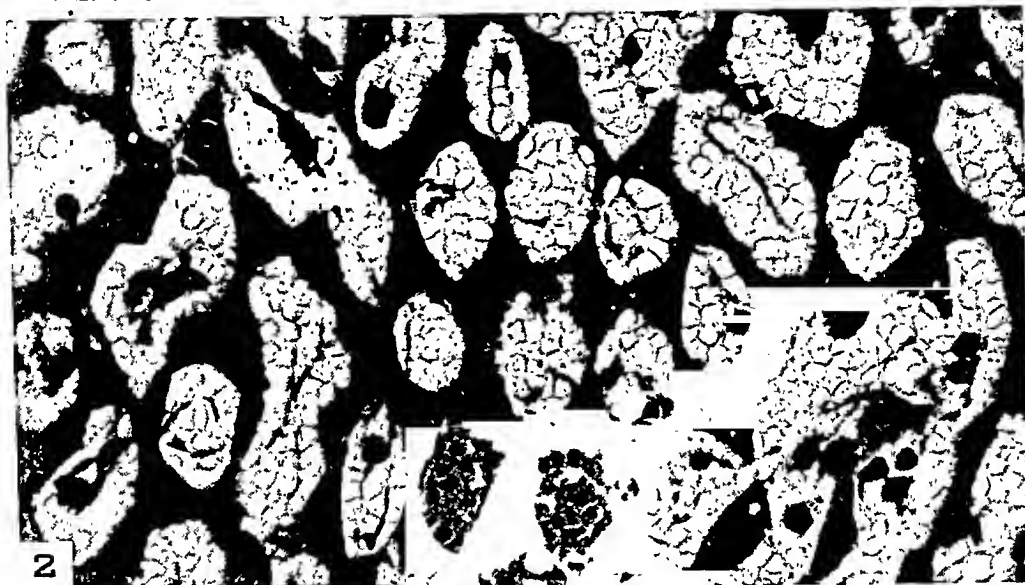
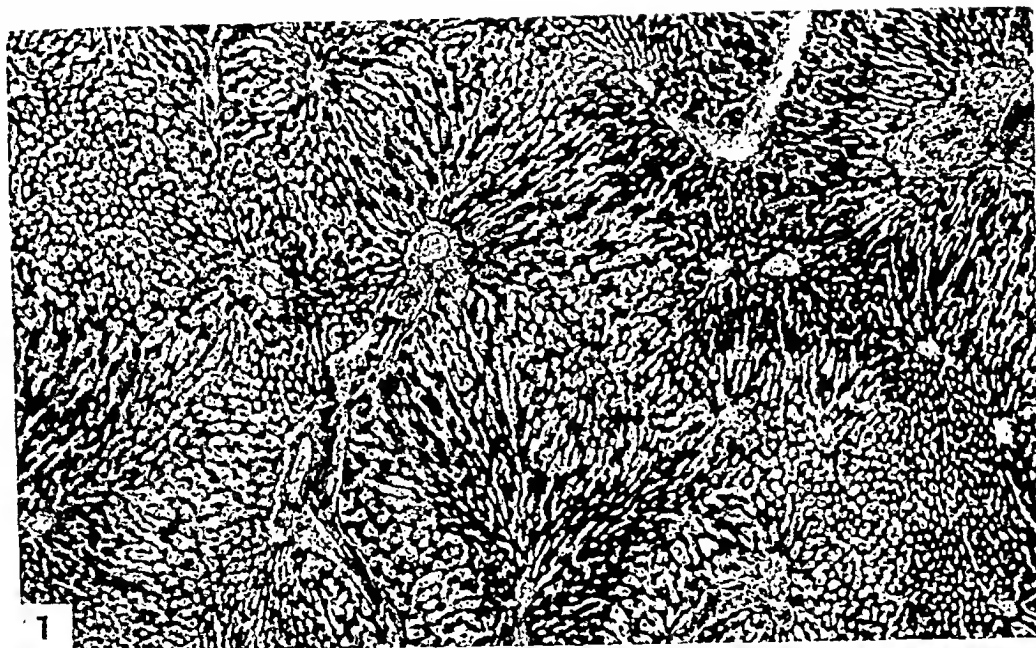
### DESCRIPTION OF PLATES

(All figures were taken from hematoxylin and eosin preparations.)

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#### PLATE 72

FIGS. 1-3. All from rat 83, which was killed 2 hours after the administration of allyl formate. Extensive sinusoidal congestion and rupture of capillaries. Figure 1,  $\times 59$ ; Figure 2,  $\times 580$ ; Figure 3,  $\times 1400$ .



Rosin and Doljanski

Rat's Liver in Allyl Formate Poisoning

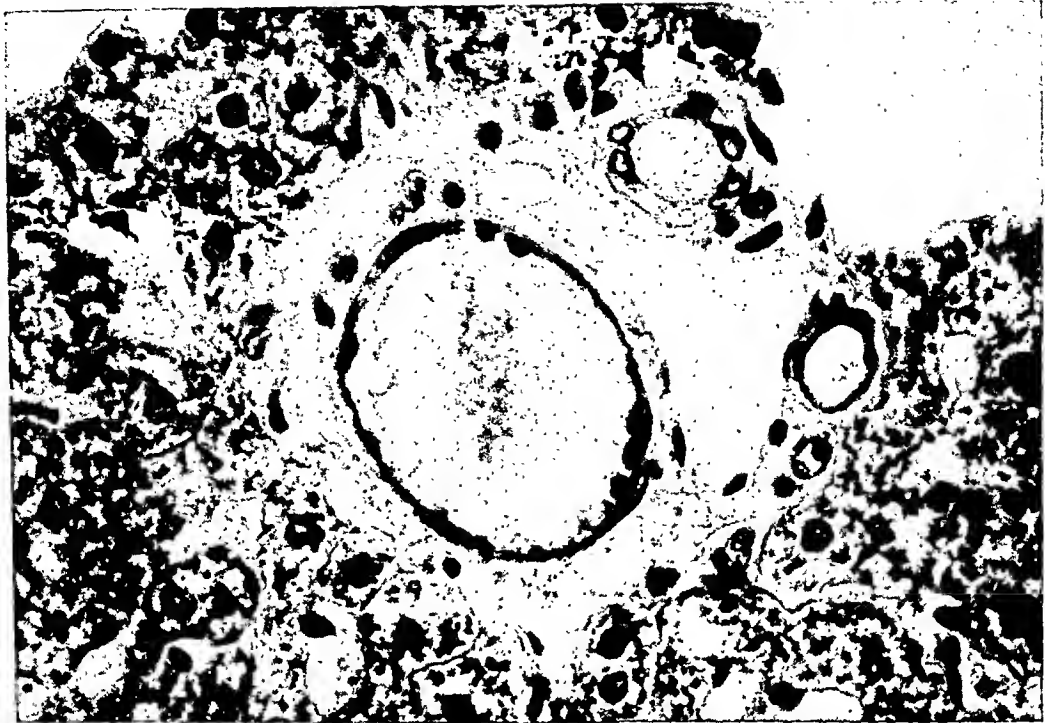
PLATE 73

FIG. 4. From rat 70, which died 1 hour after the administration of allyl formate. Marked edema around a portal vein.  $\times 620$ .

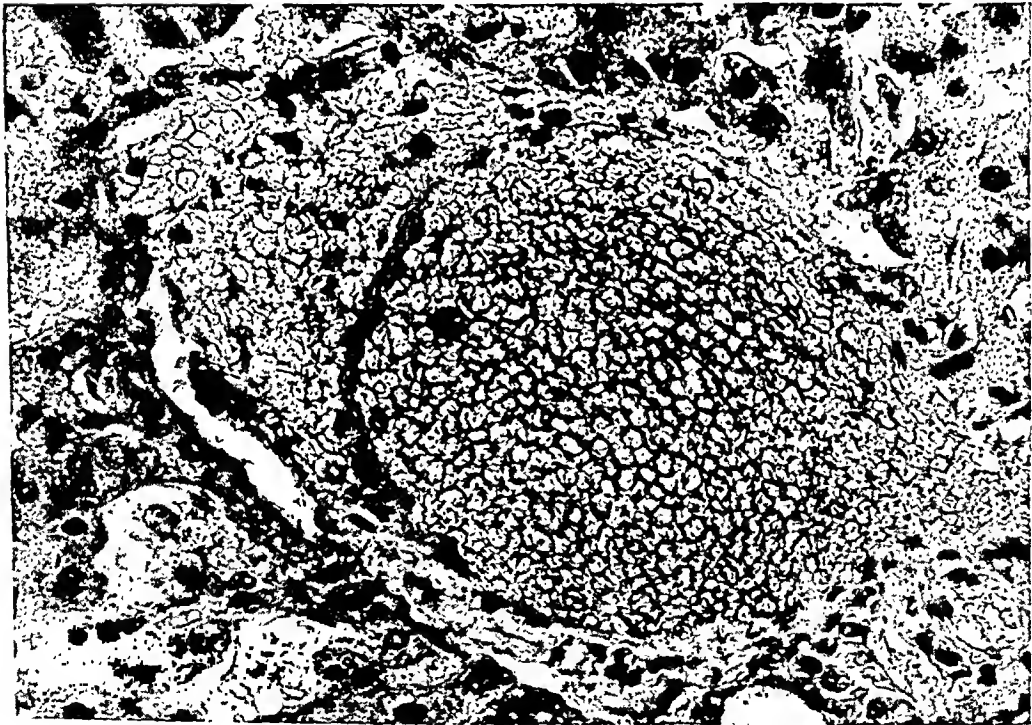
FIG. 5. From rat 78, which died  $1\frac{1}{2}$  hours after the administration of allyl formate. Hemorrhage about a portal vein.  $\times 500$ .

FIG. 6. From rat 76, which died 1 hour after the administration of allyl formate. Beginning hydropic degeneration of the hepatic cells; the vacuoles contain homogenous pinkish-staining material; in addition, in one liver cell (c) there is a single erythrocyte.  $\times 1700$ .

4



5



6



PLATE 74

FIG. 7. From rat 17, which died 6 hours after the administration of allyl formate. Periportal area with extensive damages. The sinusoidal capillaries are broken up and the liver cells are swollen and contain large vacuoles filled with erythrocytes.  $\times 170$ .

FIG. 8. Higher magnification of an area in Figure 7.  $\times 800$ .



7



8

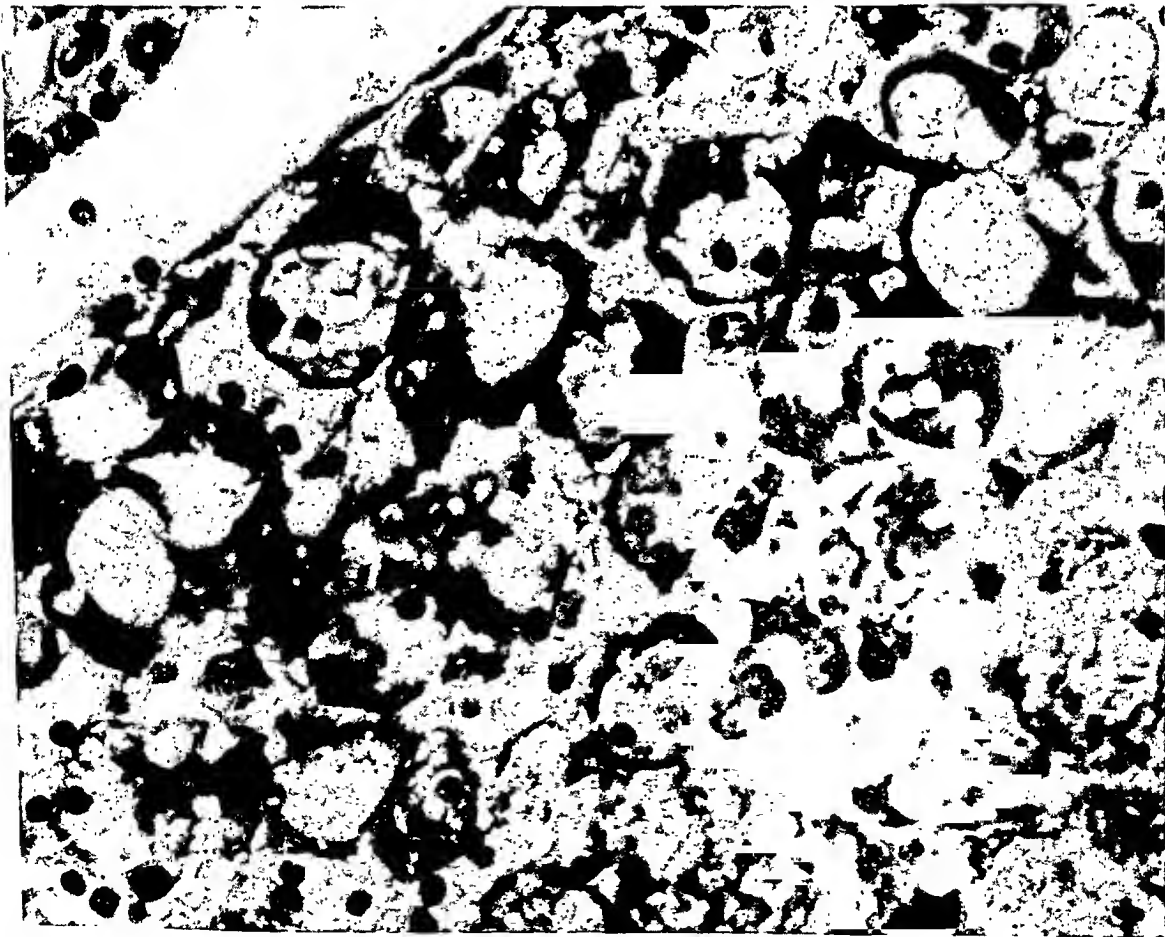
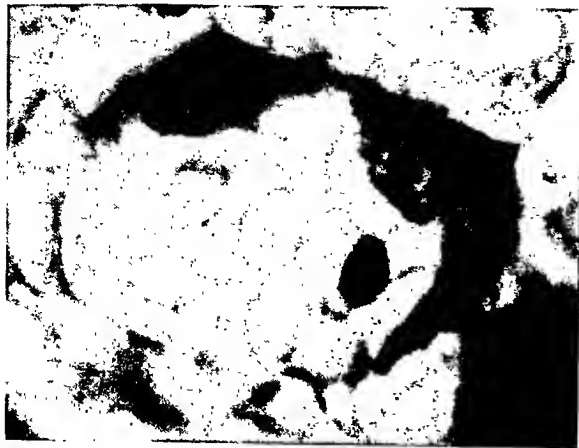




PLATE 75

FIGS. 9-13. All from the same animal as Figure 7. Erythrocytes in liver cells.  
× 1600 to 1900.

9



10



11



12



13



Rosin and Doljanski

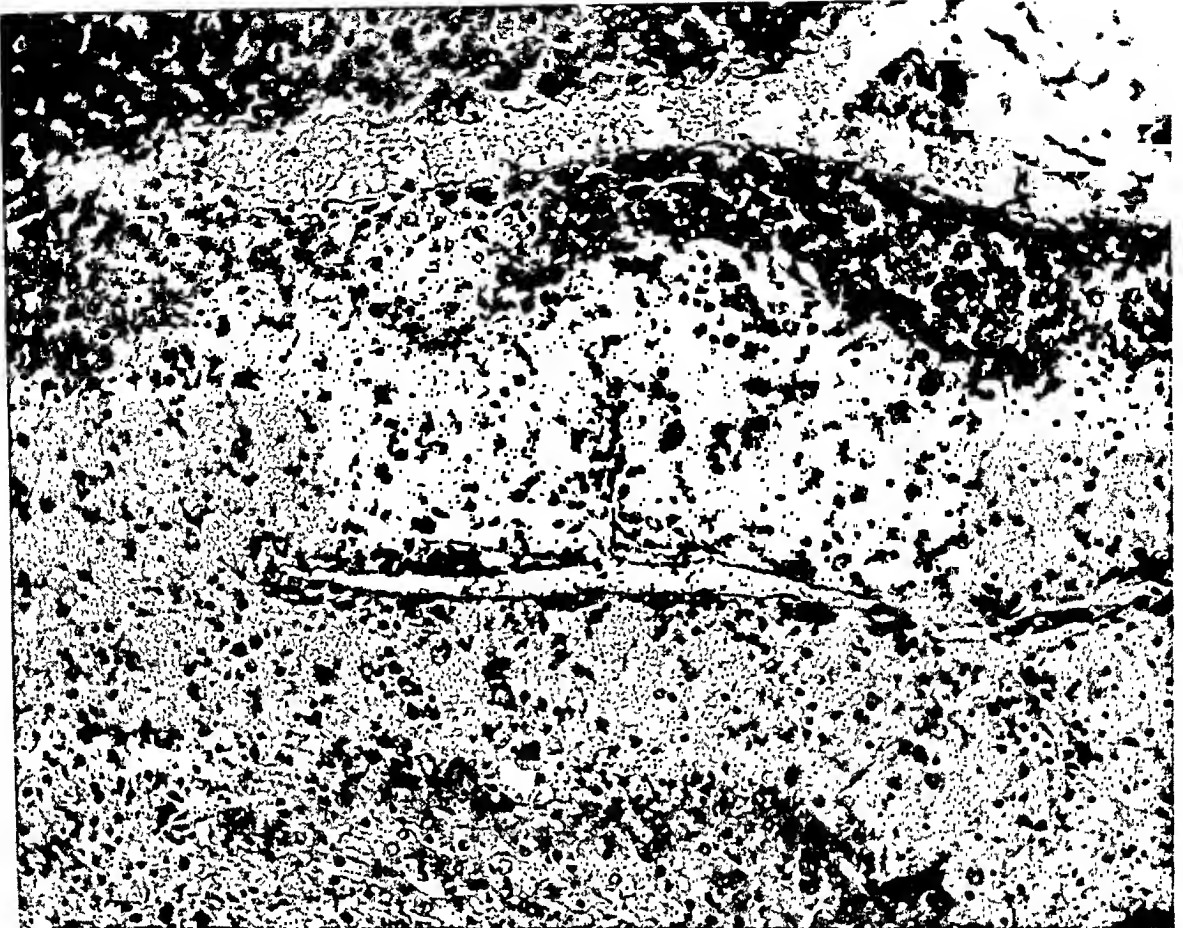
Rat's Liver in Allyl Formate Poisoning

PLATE 76

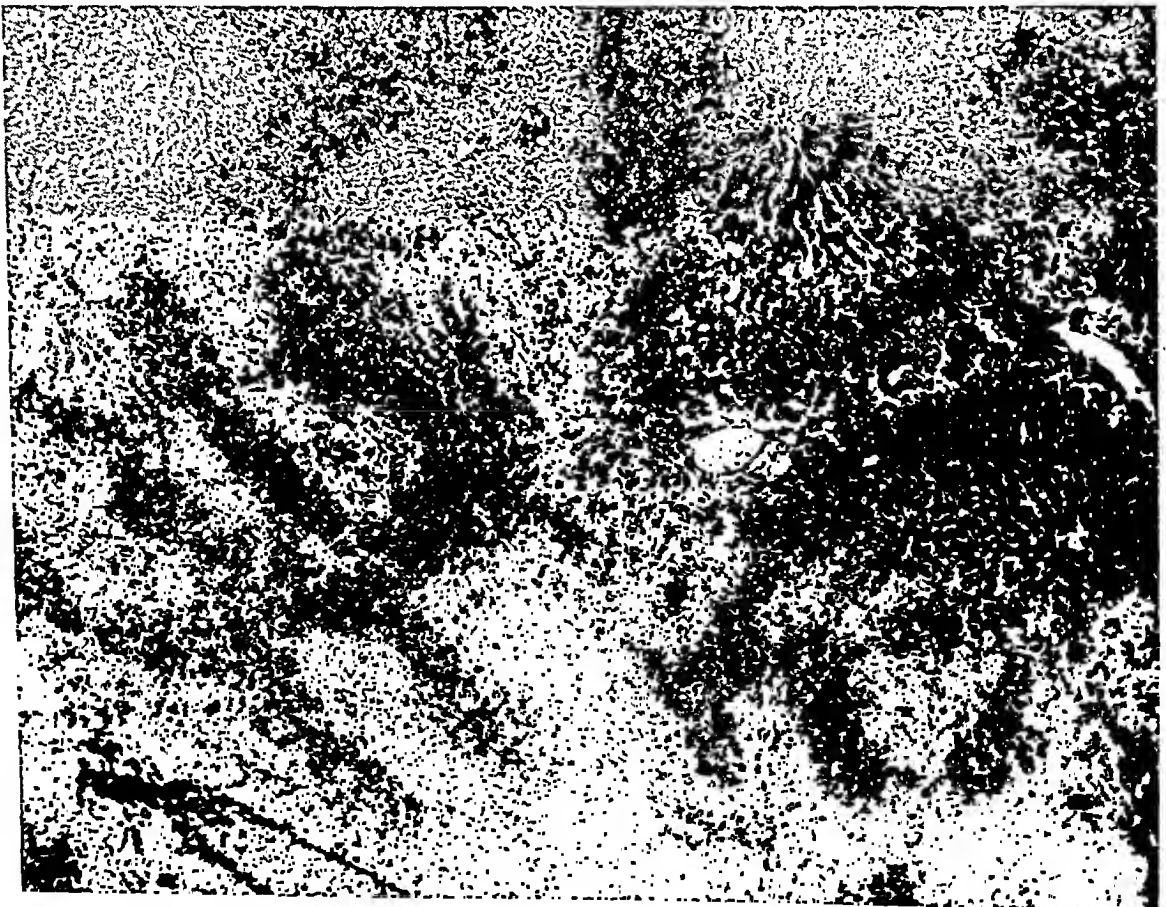
FIG. 14. From rat 20, which died 4 hours after the administration of a second dose of allyl formate. Extensive hemorrhages.  $\times 169$ .

FIG. 15. From rat 18, which died 7 hours after the administration of a second dose of allyl formate. Zones of necrosis.  $\times 72$ .

14



15





# PRIMARY TUMOR OF THE HEART CONTAINING EPITHELIUM-LIKE ELEMENTS \*

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Primary tumors of the heart, although rare, have excited such interest among those by whom they have been encountered that more than 100 cases have been reported. These cases have been collected and reviewed from time to time,<sup>1-8</sup> so no attempt will be made here to survey these reports completely.

In reviewing the descriptions of reported cases, one is struck by the fact that, with relatively rare exceptions, most of the primary cardiac tumors form a well defined group. Characteristic of this group of tumors are an almost constant origin from one atrium or the other and commonly from the interatrial septum, polypoid projection of the tumor into the cavity of the atrium, associated thrombosis, and a microscopic structure predominantly of myxomatous or angiomatous nature. Both benign and malignant forms occur. The terminology used in reporting these tumors is indicative of variable histologic structure and interpretation. The benign forms have been termed myxoma, fibromyxoma, hemangioma, hemangio-endothelioma, lymphangioma, lymphangio-endothelioma, pseudomyxoma, fibro-angiomyxoma, and hemangio-elastomyxoma. Malignant representatives that appear to fit in this group have been called myxosarcoma, fibromyxosarcoma, angiosarcoma, and angioretico-endothelioma (Kaposi's disease).

The majority of these tumors have been found in the right or left atrium, appearing to have originated from the atrial septum near the fossa ovalis, although other origins, as from the pulmonary artery,<sup>9</sup> have been described. The relative constancy of the site of origin of this group of tumors suggests that it has some significance. Ribbert<sup>9</sup> maintained that rests of embryonic mucoid tissue may persist in the heart, especially in the rim of the foramen ovale. Certainly the tendency to a particular site of origin is suggestive of derivation from some type of embryonic rest, in analogous fashion to the origin of certain other tumors.

It is not surprising that any tumor involving endocardium and projecting into the cardiac cavity should be associated with thrombosis. Debate has arisen, however, over the possibility that formation of the thrombus may be primary, with neoplasm developing in the thrombus.

\* Received for publication, March 19, 1945.

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Indeed, it has been maintained that some of the benign forms may not be of neoplastic nature, but simply thrombi which have undergone a myxomatous type of degeneration.<sup>10</sup> Warthin<sup>11</sup> described a mucoid fibroblastic tissue as a common result of organization of cardiac thrombi, and was unconvinced that a true myxoblastoma of the heart existed. However, it is not possible to doubt that truly neoplastic polypoid tumors containing a myxomatous tissue have been found in the heart. It may be, though, that the myxomatous element is a degenerative change in the stroma, or a result of a change in associated thrombotic material, rather than a truly neoplastic component. The arguments favoring the genuine neoplastic nature of at least some of these tumors have been considered in several papers<sup>5,12</sup> and need not be repeated here.

The following case appears to belong to this group of tumors, having the characteristic position and structure, with prominence of myxomatous change and abundant proliferation of small vascular channels. It is unique, however, in that included in it are cells which appear to be of epithelial or mesothelial nature, and which form gland-like cystic structures.

#### REPORT OF CASE

A. H., a white woman, 53 years of age, came to the hospital because of shortness of breath and swelling of the feet and legs. These symptoms had not been severe enough to interfere with her work until 4 weeks previously. No history of rheumatic fever was elicited. The findings were those of severe congestive cardiac failure, with auricular fibrillation and evidence of aortic regurgitation and stenosis. Symptoms increased in severity, and death occurred approximately 1 week after admission.

Post-mortem examination showed the anasarca and chronic congestive changes of viscera commonly associated with failure of the circulation. The unopened heart weighed 790 gm. The right atrium contributed largely to the increased size and weight of the heart, for it showed a bulky, rounded, firm enlargement to a diameter of 6 cm. The aortic valve was stenotic, with thickened, shortened, and adherent, calcified leaflets.

The distention of the right atrium was due to an egg-shaped tumor mass, measuring 8 by 5 cm., which filled and enlarged this portion of the heart. The upper and main portion of the tumor was an oval mass, 4 cm. in diameter, tightly enclosed on all except its lower end by the stretched wall of the atrium. This enclosing capsule of atrial wall was thinned out in its upper part to 1 mm. and even less in some regions. In some areas it was possible to strip away the tumor from the atrial wall along a line of cleavage, but in other parts, and particularly near the summit of the mass, the neoplastic tissue blended with the wall so that separation could not be accomplished without tearing.

Projecting downward from the main body of the tumor and hanging free above the tricuspid valve were some irregular nodular projections of the tumor mass. This free lower end was covered by a thin, smooth, grayish blue membrane which was continuous with the endothelial lining of the atrium. In some areas where this covering membrane was deficient, reddish brown nodules of friable material projected through the ulcerations. These were evidently masses of thrombotic material.

Sections through the tumor showed that the main bulk was composed of a firm, rubbery, highly translucent tissue of grayish color. This rubbery tissue was interrupted by some irregular white areas, more fibrous in appearance and consistency. Occasional small blood vessels, of a size just visible by macroscopic examination, and containing clotted blood, could be discerned.

In various parts of the tumor there was a honeycomb structure due to irregular, small, cyst-like spaces, measuring up to  $1\frac{1}{2}$  cm. in diameter, and filled with mucoid material of jelly-like consistency. Removal of this material showed that each cyst had a smooth, white, membrane-like lining. In other areas there was a more angiomatous appearance, with thin-walled spaces filled by dark-red clotted blood.

The lowermost portion of the tumor mass, particularly where the limiting membrane was deficient, was composed of brown and gray friable tissue, contrasting with most of the tumor in both color and consistency, evidently thrombotic material. This was separable from the rest of the tumor, and nowhere did the thrombus and the tumor tend to be firmly adherent or imperceptibly to merge, but rather there seemed to be a line of cleavage.

The microscopic structure of the tumor showed marked variation in different areas. Most of the tissue had a myxomatous appearance, being composed of relatively few cells set in an abundant, loose, pale-staining stroma. In some areas the stroma stained well with eosin, and had a homogeneous colloid-like appearance. In other regions the stroma was faintly basophilic or eosinophilic, and had a loose fibrillar structure most striking in appearance around the fairly numerous capillaries. Sparsely situated in this abundant stroma were a few small cells scantily arranged in short lines of cells to give a spider-like appearance.

Other areas were highly cellular, with scanty stroma, and an entirely different appearance. These highly cellular areas had a rather sarcomatous aspect, and were supplied by moderate numbers of thin-walled capillary vessels. Where these cellular areas bordered on myocardium, there was invasion between individual muscle cells and around bundles of the myocardial fibers, with associated degenerative changes and atrophy of the parenchymatous tissue. In some areas there was invasion through the muscle of the auricular wall and involvement of epi-



cardial fatty tissue. The component cells were small, with oval nuclei and scanty cytoplasm having indefinite outlines. Fairly numerous plasma cells and basophils were mixed with the tumor cells, particularly in the less cellular areas. Fibers stainable by silver impregnation were not associated with these cells. No transition from myocardial fibers was detectable nor could any evidence of striations be found. Mitotic figures were seen very infrequently.

In some small areas lymphocytes only were present, to the exclusion of other types of cells, with no evidence of follicle formation. Again, in other regions, small, thin-walled blood vessels were so abundant that the tissue had a hemangiomatous appearance. There were occasional areas of bone formation.

Glandular cyst-like spaces lined by epithelium-like cells were evident in various areas of the tumor. These varied from tiny gland-like structures to the larger cystic spaces which were evident grossly. The epithelium was usually in a single layer and varied from flat cells of endothelial appearance to tall columnar cells, closely placed, and with regular basal nuclei. In some areas the epithelial cells showed evidence of secretory activity. Some of the epithelial linings showed papillary infoldings, or nodular areas several cells in thickness. Amorphous material and desquamated cells formed the contents of the cystic spaces. The material in some of the cavities and within vacuoles in the lining cells stained with mucicarmine. Some of the lining cells had frayed inner margins suggestive of cilia, although nowhere was this definite enough to be unquestionable.

Deposition of pigment was common throughout various parts of the tumor. This was mostly in the form of brownish granules of hemosiderin pigment, which gave a Prussian-blue reaction. A curious finding was the staining of connective tissue trabeculae by bluish-black iron-containing pigment in a fashion similar to that seen in the spleen in sickle cell anemia. In several areas there were actual siderofibrotic nodules (Gandy-Gamna bodies), with bamboo-like segmented rods of yellowish and brownish color which simulated the mycelial threads of fungi.

#### DISCUSSION

The position, the gross appearance, and the angiomatous and myxomatous microscopic character of this tumor clearly places it with the commonly described group of primary cardiac tumors. The unique feature lies in the inclusion of elements of epithelial appearance, which hitherto have not been described in such tumors. In position, the myxomatous and angiomatous portions of the tumor mass were in close association with thrombotic material, but had no constant relationship to

the epithelium-like structures. The appearance and relationships of the myxomatous and angiomatous elements give the impression that they are due to changes developing in a thrombus, possibly promoted by the mechanical forces brought into play by the peculiar position.

The explanation of the origin of the epithelium-like structures is not readily apparent. The presence of abundant channels and spaces lined by endothelium suggests the possibility of origin from such endothelium by a metaplastic change to cells having the appearance of epithelium. This possibility receives some support from the observation that in the lining of some of the cyst-like spaces a transition can be observed from flat cells resembling endothelium to tall columnar cells with basal nuclei. However, no evidence was found that any space having an epithelium-like lining was actually of vascular origin, from either content, structure of the wall, or connection with a definite blood or lymph channel. Vascular endothelium is not known to have potentialities for transition to tall columnar cells of epithelial character.

A more probable origin would seem to be from the inclusion of cell rests having potentialities for forming structures of this type. The striking constancy of origin of primary cardiac tumors of myxomatous or angiomatous nature from the region of the interatrial septum suggests the probability that they arise from some developmental abnormality or inclusion. Rezek<sup>13</sup> has described a tumor of microscopic proportions found beneath the endocardium of the right atrium and enclosed entirely within the interatrial septum. This tumor had a structure which linked it with the more bulky tumors which project into an atrial cavity, and it also contained elements of epithelial appearance. It may be, as he suggested, that such structures arise from epithelium displaced in the heart during early stages of embryonic development. As has been pointed out,<sup>14</sup> during early embryonic development there is an opportunity for heterotopic inclusion of entodermal tissue of the primitive foregut in the mesodermal tissue which forms the heart. The mucinous production in the tumor reported here would be consistent with such an entodermal origin. Some of the rare epithelial cysts of the heart<sup>14-18</sup> have been explained on this basis, and it would seem possible that a similar epithelial inclusion may have been the origin of the present tumor. A hypothesis of origin from a heterotopic inclusion is difficult to substantiate and equally difficult to refute. It is almost as easy to suggest that the heterotopic cell inclusion was of an even earlier stage and possessed potentialities for forming the epithelial and the mesenchymal structures evident in the tumor, and that hence it should be regarded as a mixed tumor or teratoma.

It must be considered as a possibility that this woman had a primary

carcinoma of the alimentary tract which remained small and could not be found by the usual autopsy procedures, but which nevertheless metastasized to the heart. For this there is lack of any suggestive evidence, and it may be dismissed as highly improbable.

Finally, the possibility of pericardial origin must be considered. Mesoblastic lining cells, such as those of the pleura and pericardium, have potentialities for forming epithelium-like structures<sup>18</sup> similar to those in the case described here, and serous epicardial cells have often been observed to differentiate into tall columnar cells.<sup>14</sup> These mesoblastic cells are capable of forming a mucoid substance. Some of the cystic structures found in the heart have been thought to have an epicardial origin, due to invagination of the outer part of the myocardial plate, which forms the epicardium, into the inner plate from which the myocardium is formed.<sup>19</sup> A pericardial origin seems to be favored by the marked variation in the form of the cells lining the cysts, transitions from low flattened cells to tall columnar cells being observable. However, extension of the tumor cells through the thin atrial wall with microscopic evidence of involvement of epicardium probably cannot be considered as signifying direct origin from pericardial tissue.

While inclined to feel that the evidence favors a pericardial origin for the epithelium-like structures of the tumor, we realize that considerations as to origin are speculative and inconclusive at present.

### CONCLUSION

A primary myxomatous cardiac tumor arising from the right atrium is described. A unique feature of this tumor is the presence of epithelium-like cells forming gland-like and cystic spaces. The possible origins are discussed, and the conclusion reached that it is probably due to the inclusion of pericardial elements in the atrial wall during cardiac development. The myxomatous and angiomatous features of the tumor are believed to be the result of changes in associated thrombotic material.

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[ Illustrations follow ]

## DESCRIPTION OF PLATES

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### PLATE 77

- FIG. 1. External appearance of the heart, showing the bulge of the wall of the right atrium produced by the tumor.
- FIG. 2. Cut surface of the tumor, showing the upper portion blending with the atrial wall, and the lower part free over the valve. Cystic areas are evident on the cut surface.
- FIG. 3. Myxomatous area of the tumor.  $\times 150$ .
- FIG. 4. Region of the tumor showing a portion of the lining of two cystic spaces, other gland-like structures, and cellular infiltration around remnants of muscle fibers.  $\times 120$ .

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2



3



4



Anderson and Dmytryk

Primary Tumor of the Heart

PLATE 78

FIG. 5. A region of the tumor showing several gland-like spaces and cystic areas.  $\times 300$ .

FIG. 6. A portion of the wall of one of the cysts, having the appearance of secretory activity.  $\times 550$ .

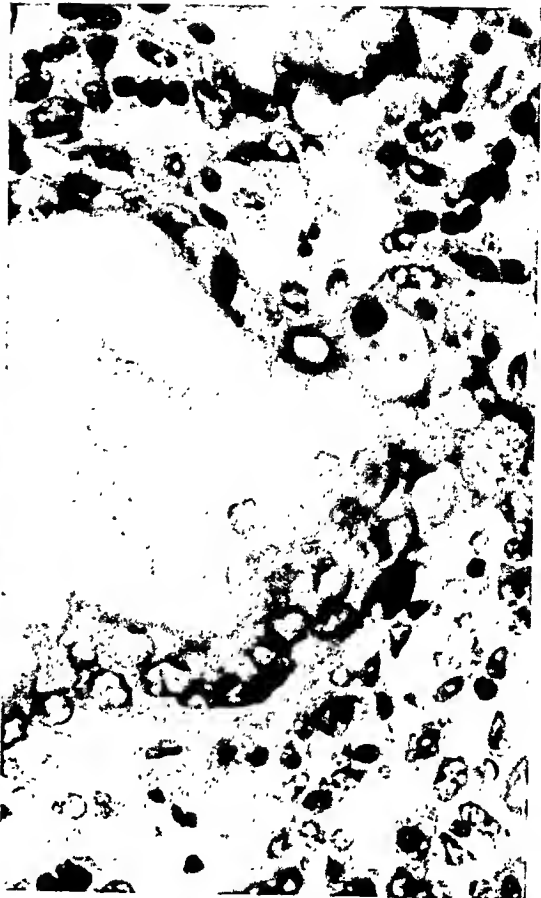
FIG. 7. A portion of the lining of one of the larger cysts, showing columnar cells with frayed inner margins.  $\times 750$ .

FIG. 8. An area of a cyst wall in the tumor, showing columnar lining cells with basal nuclei.  $\times 300$ .

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Anderson and Dmytryk

Primary Tumor of the Heart

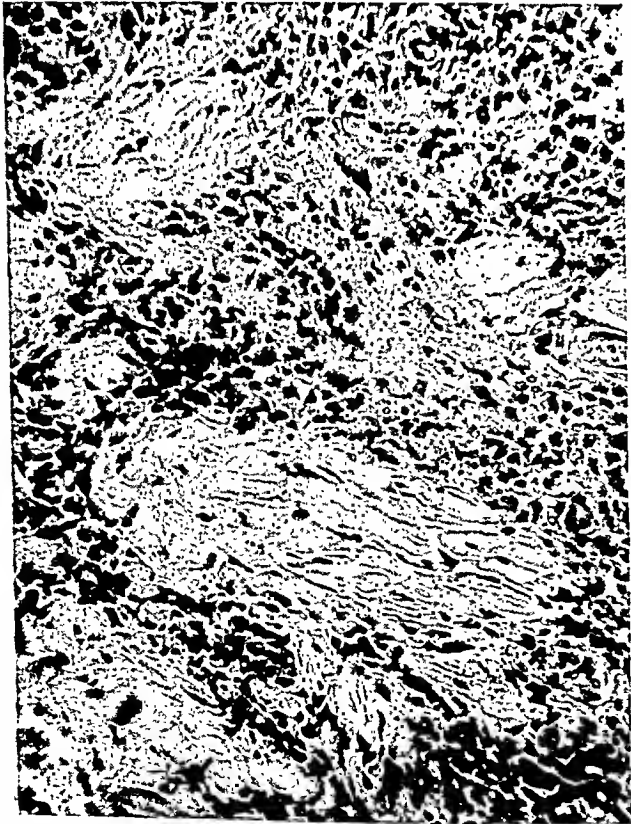


PLATE 79

FIG. 9. Bone and marrow formation in the tumor, and some epithelium-like elements.  $\times 120$ .

FIG. 10. Remnants of myocardial fibers surrounded by the small, dark cells which composed most of the tumor.  $\times 550$ .

FIG. 11. An area of pigment accumulation, showing impregnation of fibers.  $\times 150$ .



Anderson and Dmytryk

Primary Tumor of the Heart



# GROSS VASCULARITY OF THE MITRAL VALVE AS A STIGMA OF RHEUMATIC HEART DISEASE \*

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Grossly vascularized mitral valves are usually the seat of rheumatic endocarditis, *i.e.*, mitral stenosis or nondeforming valvular disease. Sometimes, however, the vascularized leaflets appear normal in the gross and no conclusive rheumatic stigmas are present elsewhere in the heart. This study deals with the morphology of vascularized mitral valves in hearts with and without gross rheumatic disease.

## METHOD

Selection of hearts was based on diffuse vascularity of the anterior mitral leaflet, extending from attachment to line of closure. Vascularity of the posterior mitral leaflet, although present in many instances, was not as well marked and was often focal and of variable pattern. Hearts with vascularization limited in the gross to the basal third or half of the anterior leaflet were excluded.

The study comprised 150 hearts with grossly vascularized anterior mitral leaflets, divided into three groups as follows: *Group 1*, 50 hearts with no conclusive gross rheumatic disease; *Group 2*, 50 hearts with nondeforming rheumatic mitral disease; *Group 3*, 50 hearts with mitral stenosis.

In group 1 no gross stigmas of rheumatic disease were present in the mitral valve or elsewhere in the heart. In the hearts of group 2 the mitral valve was the seat of nondeforming valvulitis, *i.e.*, opacity and thickening of the leaflets especially in the line of closure, without shortening or commissural fusion, and with thickening or fusion of chordae tendineae. The hearts in groups 2 and 3, especially those with mitral stenosis, frequently showed deforming or nondeforming disease of aortic, tricuspid, or pulmonic valves and, less commonly, chronic endocarditis of the left atrium and pericardial adhesion.

Fifty nonrheumatic adult hearts with grossly avascular valves were included as a control.

Microscopic sections, prepared from all hearts according to the method of Gross, Antopol, and Sacks,<sup>1</sup> were stained with hematoxylin and eosin. Duplicate sections were stained with the combined Weigert and van Gieson methods for elastic and connective tissue. The mitral leaflets were examined for type, location, and distribution of vessels,

\* Received for publication, April 24, 1945.

cellular exudate, and endocardial reduplications. Five other cardiac sites were examined for rheumatic stigmas, *i.e.*, the posterior papillary muscle of the left ventricle, the posterior endocardial wall of the left atrium, and the aortic, tricuspid, and pulmonic valves.

The lesions accepted as rheumatic were: *myocardium*, Aschoff nodules; *left atrial endocardium*, Aschoff nodules, subendothelial plaques, vascularity and cellular exudate of the endocardium proper, and hyper-vascularity and cellular exudate of the subendocardium; *valves*, vascularity of the free portions, especially with vessels of arterial type, cellular exudate, and endocardial reduplications. Atrial and valvular stigmas frequently occurred in combination. Equivocal changes were not included as stigmas.

### DESCRIPTION OF RHEUMATIC LESIONS

Three characteristic microscopic stigmas of rheumatic heart disease are: (1) vascularization of valves with thick-walled arteries, (2) Aschoff nodules, and (3) endocardial reduplications of valves, especially mitral and aortic. A description of the first is given subsequently.

Aschoff nodules consist of typical Aschoff cells, the Anitschkow myocytes, with "owl-eyed," fibrocytoid, or pyknotic nuclei contained in a collagenous matrix which is swollen and coarsely granular. The cytoplasm of the cells is frequently basophilic and has a ragged, poorly defined peripheral border. Only typical nodules of this sort were accepted as rheumatic.

Endocardial reduplications occur in a subendothelial location, affecting the auricularis and ventricularis layers of atrioventricular valves, and the arterialis and ventricularis layers of semilunar valves. Gross and Friédberg<sup>2</sup> attributed this change to proliferation of fixed mesenchymal cells which are then transformed into connective tissue with subsequent elastification. The lesion appears to consist of an additional layer of fibro-elastic tissue superimposed on the endocardial surface of the valve. The reduplications are sometimes confined to the attachment or free portion of the valve, but may be diffuse, extending from the base of the leaflet well out toward the line of closure. The valvular attachment is a common site. Here, involvement of the ventricularis or arterialis layer of the aortic valve produces the so-called subaortic angle or pocket lesion respectively, while involvement of the ventricularis layer of the mitral valve results in the subvalvular pocket lesion. Reduplications may be vascularized, especially with thick-walled small arteries, may occasionally contain smooth muscle cells, and sometimes show cellular exudate, scarring, or calcific deposit. Although generally com-

posed of only one layer of connective tissue, there are reduplications of multiple type which consist of two or more distinct layers separated by a dense elastic membrane. Rheumatic reduplications are occasionally difficult to distinguish from similar changes in nonrheumatic hearts. Gross and Friedberg<sup>2</sup> pointed out that since collagenous thickening and elastification of valvular layers usually occur normally with increasing age, reduplications should not be attributed to rheumatic disease unless they are beyond the normal range or show qualitative differences such as vascularity or cellular exudate.

### *Morphology of the Vessels in Vascularized Mitral Valves*

*Gross Description.* A fairly detailed account of the gross appearance of vascularized valves was given by Ribbert.<sup>3</sup> In the present study the vascularization of mitral leaflets was generally similar in the hearts of groups 1 and 2. In mitral valves, the seat of stenosis, the vascularity was often obscured because of thickening and opacity of leaflets.

The vessels extended from the valvular attachment to the line of closure and varied from few to an extensive branching network with numerous anastomoses. Variation in pattern was much more frequent in the posterior than in the anterior leaflet. In the latter there were often one or two main arterial stems at the base of the leaflet, occasionally more than two, arising in the lateral thirds, especially adjacent to the posterior commissure, and less commonly in the central third of the base (Figs. 1 to 4).

The vessels were delicate in appearance or formed prominent cord-like structures situated just beneath or projecting slightly upon the endothelial surface of the leaflet on the atrial side. They were usually readily visible to the naked eye, especially when filled with blood. Sometimes they were easier to visualize when the valve was viewed obliquely or by transmitted light.

*Microscopic Examination.* The structure of the vessels in both leaflets of the mitral valve was generally similar in practically all hearts of groups 1, 2, and 3. The vessels were sectioned in longitudinal, transverse, and oblique planes.

The leaflets contained capillaries, arterioles, and arteries; veins were probably present but were difficult to distinguish from the arteries. The vessels were situated on the atrial side of the valve, were frequently subendothelial, and might project upon the surface of the valve. In the anterior leaflet they occurred mainly in the auricularis layer; involvement of the spongiosa was less conspicuous and of fibrosa and ventricularis, rare. In the posterior leaflets the vessels were situated

in the spongiosa and auricularis layers and occasionally in the fibrosa and ventricularis. At the line of closure and tip of the valve the vascularity was often abundant and diffuse.

The predominant vessel was a small artery with thick musculo-elastic wall (Figs. 5, 6, and 7). Although this usually measured 150 to 250  $\mu$  in transverse diameter, there was a wide range from about 50 to 500  $\mu$ . Most vessels of arteriolar size, *i.e.*, less than 75  $\mu$  in diameter, conformed to this type (Fig. 8). Aside from capillaries and a small number of arterioles, there were very few, if any, vessels of normal appearance.

The structure of the musculo-elastic artery differed substantially from the normal small artery. The vessel was composed principally of smooth muscle cells orientated longitudinally instead of in the customary transverse manner, and partly separated by elastic fibers (Fig. 9). On transverse section the cytoplasm of the muscle cells was often clear or vacuolated so that the vessel wall had a honeycombed appearance. The lumen was generally narrow, might be eccentric, and was lined by endothelium which appeared to rest directly on muscle. A thin circular layer of muscle, a few cells in thickness, was frequently present at the periphery of the vessel, but this was rarely well developed. A clear-cut adventitia was not discernible.

Some vessels conformed to the so-called intimal musculo-elastic artery described by Gross, Kugel, and Epstein<sup>4</sup> as characteristic of rheumatic heart disease (Fig. 10). Here, as shown most clearly by section in the long axis of the vessel, the longitudinal muscle was confined to the region of the intima and was surrounded by a transversely arranged media. However, in most musculo-elastic vessels it was difficult to identify intimal and medial layers, either in longitudinal or transverse section, because of the variable disposition of the elastica.

A clear-cut internal elastic lamina was usually absent. Sometimes a fairly well defined, circular, elastic membrane, single or reduplicated, was present, but this varied in location, *i.e.*, adjacent to endothelium, or at the outermost margin of the vessel, or between these sites. Accordingly, the longitudinal muscle cells lay entirely within, or external to, or on both sides of this elastica. Occasionally there were two separate elastic laminae, usually situated near the endothelium and at the outer border of the vessel respectively. The musculo-elastic vessels often resembled the *état réticulaire* of Rabé<sup>5</sup> but the latter is a distinct lesion of the medial layer resulting from degeneration and vacuolization of muscle cells.

The elastic component of the arteries often underwent progressive change in amount and configuration. The original network of delicate

fibers was condensed and rearranged to form single or reduplicated circular laminae; then, through thickening and further reduplication, the elastica formed concentric bands extending through the entire wall (Fig. 11). Associated with this process were deposition of collagen, and atrophy of muscle cells with connective tissue replacement. The end-result was an artery of fibro-elastic type. The time relations of these progressive changes are not clearly known. It has been suggested that fibro-elastic metamorphosis may occur within 1 year.<sup>2</sup>

A summary of the findings is given in Tables I to IV.

TABLE I  
*Distribution of Musculo-Elastic Vessels in 150 Hearts with Grossly Vascularized Mitral Valve*

	Number of cases	Anterior mitral leaflet	Posterior mitral leaflet	Posterior papillary muscle of left ventricle	Left atrium	Aortic valve	Tricuspid valve	Pulmonary valve
Group 1: Hearts with no conclusive gross rheumatic disease	50	44	38	0	2	6	2	0
Group 2: Hearts with non-deforming rheumatic mitral disease	50	48	46	1	1	11	5	1
Group 3: Hearts with mitral stenosis	50	48	44	1	4	25	16	6
Totals	150	140	128	2	7	42	23	7

*Vascularity of the Mitral Valve.* The anterior mitral leaflets of all hearts in groups 1, 2, and 3 were vascularized diffusely, usually to the line of closure or tip; this leaflet contained vessels of musculo-elastic type in 44, 48, and 48 hearts respectively in the three groups (Table I). The posterior mitral leaflet was vascularized in 46, 49, and 48 hearts and musculo-elastic vessels were found in 38, 46, and 44 hearts respectively in groups 1, 2, and 3 (Table I). In the cases of mitral stenosis or nondeforming mitral disease, vascularity of this leaflet generally extended to the line of closure, although in a few instances the vessels were apparently confined to the basal third or half. Of the 46 vascularized leaflets in hearts with no gross rheumatic disease, the vessels were confined to the basal third or half in 20 cases and extended beyond the basal half, usually to the line of closure, in 26 cases.

*Cellular Exudate of the Mitral Valve.* In 26, 36, and 41 hearts respectively of groups 1, 2, and 3 (Table II) cellular exudate of the



mitral valve was present. The lesion generally involved both mitral leaflets but in a few instances it was confined to one. The cells were principally lymphocytes, their number small, and the distribution focal. However, in several hearts with mitral stenosis the exudate was abundant and diffuse.

*Endocardial Reduplications of the Mitral Valve.* Endocardial reduplications of the mitral valve were observed in 23 mitral valves of group 1, 28 of group 2, and 40 of group 3 (Table II). In the hearts with mitral stenosis, the reduplications were well developed, often multiple and vascularized, and contributed to the thickness of the leaflets. In groups 1 and 2, however, the lesions were less well developed and were frequently excluded because they were equivocal; the

TABLE II  
*Microscopic Lesions of Mitral Leaflets in 150 Hearts with  
Grossly Vascularized Mitral Valve*

	Number of cases	Endocardial reduplications	Cellular exudate
Group 1: Hearts with no conclusive gross rheumatic disease	50	23	26
Group 2: Hearts with nondeforming rheumatic mitral disease	50	28	36
Group 3: Hearts with mitral stenosis	50	40	41
Totals	150	91	103

changes generally involved the attachment and the basal portion of posterior leaflets and were uncommon in anterior leaflets.

*Stigmas of Rheumatic Disease.* Table III gives the number of hearts showing rheumatic stigmas at each of five sites, namely, the posterior papillary muscle of the left ventricle, the left atrium, and the aortic, tricuspid, and pulmonic valves. The aortic and tricuspid valves and the left atrium were involved most frequently. The progressively higher incidence of positive stigmas in each of the three groups is probably related to the increasing severity of the rheumatic disease.

Musculo-elastic arteries occurred much more commonly in the mitral valve than elsewhere in the heart. The incidence was slightly greater in the anterior than in the posterior leaflet. Aside from the mitral valve, the most frequent sites were aortic and tricuspid valves. Involvement of the pulmonic valve, left atrium, and posterior papillary muscle of the left ventricle is uncommon or rare (Table I). The ves-

sels were occasionally observed in the myo-epicardial wedge adjacent to the valve rings.

Table IV gives the distribution of rheumatic lesions according to the number of sites involved. Involvement of three or more sites occurred in only 7 hearts of group 1, as compared to 14 in group 2 and 33 in group 3. At least one site was involved in 38, 42, and 49 hearts respectively in groups 1, 2, and 3; conversely, all five locations were

TABLE III  
*Distribution of Microscopic Stigmas of Rheumatic Disease in 150 Hearts with Grossly Vascularized Mitral Valve*

	Number of cases	Posterior papillary muscle of left ventricle	Left atrium	Aortic valve	Tricuspid valve	Pulmonary valve
Group 1: Hearts with no conclusive gross rheumatic disease	50	6	8	22	14	12
Group 2: Hearts with nondeforming rheumatic mitral disease	50	8	15	36	19	12
Group 3: Hearts with mitral stenosis	50	12	37	40	34	23
Totals	150	26	60	98	67	47

negative in 12 hearts of group 1, 8 hearts of group 2, and only 1 heart in group 3.

In 38 of the 50 nonrheumatic hearts the free portions of all valves were avascular; in 10 hearts one or more valves showed capillaries and an occasional arteriole just beyond the attachment or in the basal third of the free portion, and this was associated in 9 of the hearts with projections of the annulus fibrosis or of auricular myocardium; in 2

TABLE IV  
*Distribution of Rheumatic Stigmas According to Number of Sites Involved in 150 Hearts with Grossly Vascularized Mitral Valve*

	Number of cases	All sites negative	One site involved	Two sites involved	Three or more sites involved
Group 1: Hearts with no conclusive gross rheumatic disease	50	12	23	8	7
Group 2: Hearts with nondeforming rheumatic mitral disease	50	8	14	14	14
Group 3: Hearts with mitral stenosis	50	1	9	7	33

hearts there was a moderate number of vessels, including musculo-elastic arteries, in the basal third of the mitral valve. The last were considered probable instances of rheumatic endocarditis. Cellular exudate and well developed endocardial reduplications were absent in all valves.

### COMMENT

Hearts with diffuse gross vascularity of the anterior mitral leaflet show essentially similar microscopic lesions regardless of whether or not gross rheumatic disease is present: These lesions are identified by the type, location, and distribution of vessels in the mitral valve, cellular exudate and endocardial reduplications of the mitral valve, and the presence of rheumatic stigmas at other cardiac sites. The differences between hearts with no conclusive gross rheumatic disease and those with nondeforming mitral disease or mitral stenosis are quantitative rather than qualitative.

Hypervascularity of valves, especially with arterial vessels of musculo-elastic type and with deep penetration to the line of closure, is characteristic of rheumatic disease. Similar hypervascularity is also observed in rheumatic endocarditis of the left atrium.<sup>6</sup> The formation of new blood vessels occurs in the acute phase of rheumatic inflammation and the vessels remain as a permanent stigma when activity ceases. Gross vascularity is much more frequent in the mitral valve than in the aortic, tricuspid, or pulmonic valves.

The extent of vascularity is not necessarily proportional to the degree of valvular deformity. Some mitral valves with nondeforming rheumatic disease are more richly vascularized than are others which have become the seat of stenosis. Similarly, stenotic aortic valves are sometimes sparsely vascular in comparison with mitral or tricuspid valves which show nondeforming lesions. Moreover, while vascularity is probably the most common stigma of rheumatic endocarditis, it is not constant. Occasionally, valves with nondeforming disease and rarely even deformed valves are apparently avascular.

The new blood vessels are presumably derived from pre-existing ones situated in the atrial myocardial wedge at the attachment of the leaflets. Harper<sup>7</sup> has described the development of capillaries in acute rheumatic endocarditis by proliferating endothelial buds which acquire an adventitia from connective tissue elements within the valve. Possibly the musculo-elastic arteries are also derived by conversion of connective tissue into muscle. An alternative origin, *i.e.*, from muscle tissue already present in the valve,<sup>8</sup> is suggested by the peculiar structure of these vessels and their frequency in the mitral valve. Morphologically, the arteries resemble closely the longitudinal smooth muscle bundles

normally found in the mitral leaflets, principally in the auricularis layer where they are distributed well out toward the line of closure. In rheumatic inflammation these bundles might undergo transformation into vessels either by forming a wall around proliferating capillaries or possibly by the development of endothelium-lined channels within the muscle. Longitudinal muscle bundles are uncommon in the free portion of the normal aortic valve but are sometimes present at the attachment, especially in the subaortic angle.

The nature of the stimulus responsible for the formation of vessels is not clear. Ribbert<sup>3</sup> assumed that chemotactic substances produced by endocarditis are reabsorbed and stimulate proliferation of the endothelium of normal vessels. He pointed out that a similar process occurs in inflammatory lesions of the cornea. Harper<sup>7</sup> suggested toxic or bacterial injury to endothelium and cellular elements of the cusps. Regardless of mechanism, the evidence indicates clearly that rheumatic fever is the principal underlying cause.

Musculo-elastic arteries are observed in valves which are the seat of acute rheumatic endocarditis. Although essentially similar in structure to the chronic rheumatic lesion, the vessels usually show edema, swelling and variation of polarity of nuclei, and a minimal amount of elastica. Gross and Friedberg<sup>2</sup> claimed that a period of at least 6 weeks is required for development, a conclusion based on the absence of these vessels in 20 of 21 patients dead within 6 weeks of the first rheumatic attack. My material did not permit investigation of this point. However, the arteries were uniformly present at autopsy in the mitral valves of 15 children with acute rheumatic heart disease. Fourteen gave a history of at least one previous episode of acute rheumatic fever from several months to 5 years prior to death. Only one child had a single fatal attack and this was of 4 months' duration.

The presence in valves of musculo-elastic arteries is perhaps pathognomonic of rheumatic disease. Vessels of this type are sometimes observed in cardiovascular syphilis, but the latter is generally confined in the heart to the aortic valve where a distinction between syphilitic and rheumatic disease can usually be made without difficulty.<sup>9,10</sup> In other etiologic forms of heart disease such as those which are hypertensive and arteriosclerotic, and in cor pulmonale, valvular lesions are not produced. Vessels occur in valves with endocarditis lenta but there is commonly an underlying rheumatic basis. Gross<sup>11</sup> found that the blood vessels in the valves of 27 hearts with atypical verrucous endocarditis consisted essentially of capillaries of the granulation-tissue, endothelial-bud type; vessels with muscular walls were rarely observed except in hearts considered to have been the seat of preceding rheumatic infection. A study of 8 hearts with atypical verrucous endo-

carditis in my own material was confirmatory. At present there is no evidence that other infectious diseases, such as scarlet fever, streptococcal pharyngitis, or pneumonia, may induce formation of blood vessels in heart valves.

A control study was made of the blood vessels in such lesions as pleural and pericardial scars, arteries with canalized thrombi, and chronic inflammatory processes with the formation of granulation tissue. Of a total of 100 such cases, about one-third showed small, thick-walled arteries with a muscle component. The latter revealed a variable arrangement, either transverse or longitudinal. Musculo-elastic arteries, usually of intimal type, were found only occasionally in contrast to the frequency and predominance of these vessels in rheumatic valves (Fig. 12).

Whether human valves may normally be vascularized is still controversial. By the paraffin section method, most grossly normal valves are entirely avascular; some show capillaries and perhaps arterioles limited to the basal third of the valve where the vessels are usually contained within projections of the myocardium or of the annulus fibrosis into the free part of the leaflets.<sup>12,13</sup> Dow and Harper<sup>14</sup> reported similar results in injected hearts. Recently, however, Wearn and Moritz,<sup>15</sup> also using the technic of injection, claimed that 32 per cent of a noninflammatory group of 255 hearts showed vascularity beyond the basal third in one or more leaflets or cusps.

From the morphologic standpoint the issue is not whether normal valves are vascularized, but whether vascularized valves are normal, *i.e.*, show no evidence of endocarditis, chronic or healed. The latter can often be established clearly by means of microscopic stigmas of inflammation. Difficulty arises in borderline cases, especially those of extinct disease in which endocarditis may heal without leaving recognizable stigmas other than the vessels. In such instances, a decision as to whether the vascularity is normal or the result of inflammation may not be possible. However, the structure of the vessels alone will often provide evidence for an inflammatory basis. The presence of musculo-elastic arteries is strongly indicative of previous endocarditis. Since these vessels are in all probability acquired, valves containing them cannot be considered normal.

#### SUMMARY AND CONCLUSIONS

Hearts with diffuse gross vascularity of the mitral valve almost uniformly show microscopic stigmas of inflammatory disease, rheumatic in origin. Hence such vascularity can be regarded *per se* as an acceptable gross stigma of rheumatic heart disease.

The principal vessels in vascularized mitral leaflets are small, thick-

walled arteries or arterioles of musculo-elastic type. These consist mainly of longitudinally disposed smooth muscle cells separated by elastic fibers. In the mitral valve such vessels are characteristic, and probably pathognomonic, of rheumatic fever.

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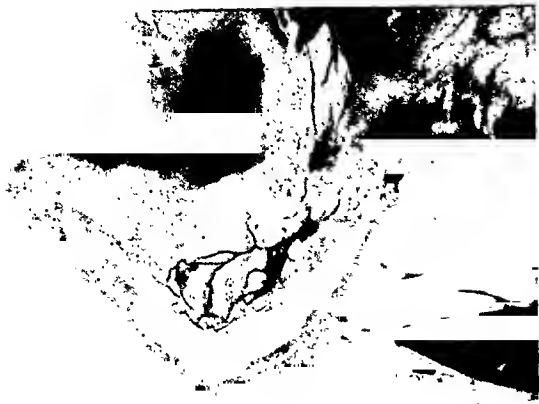
[ Illustrations follow ]

## DESCRIPTION OF PLATES

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### PLATE 80

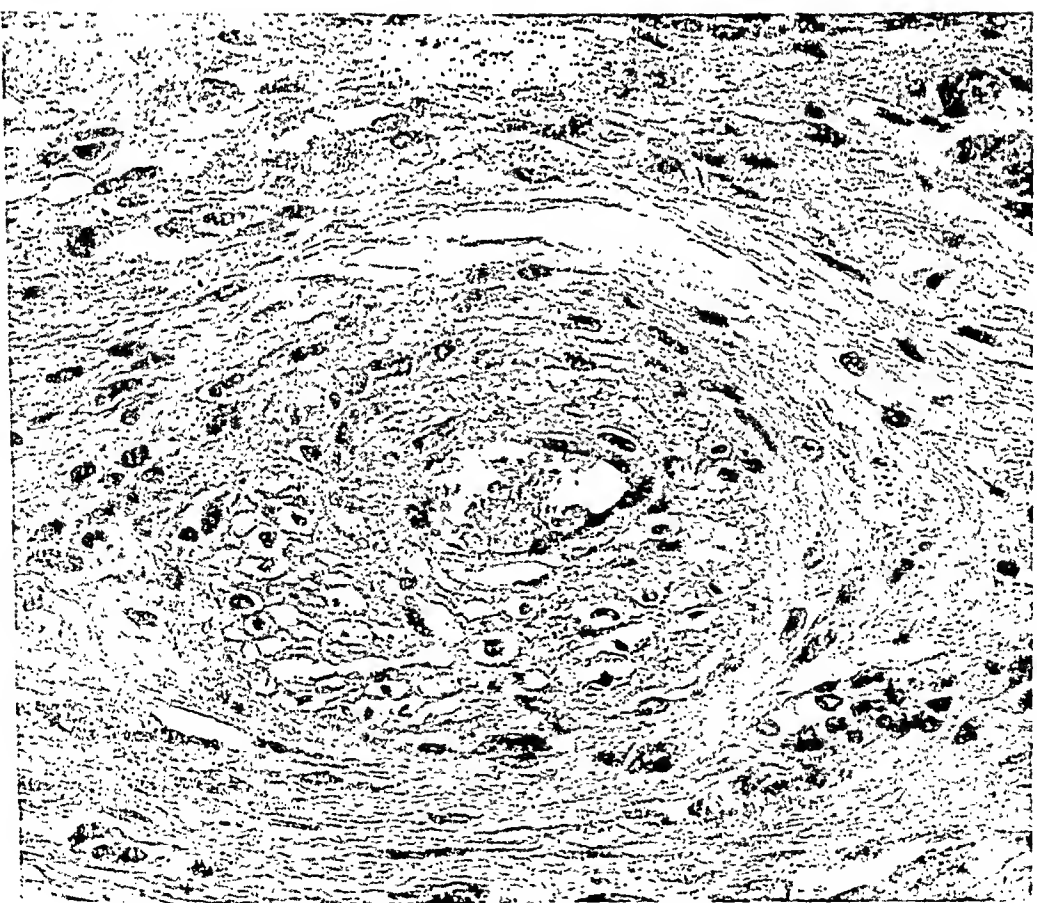
- FIG. 1. Vascularized anterior mitral leaflet in a white male, 60 years old, with no gross rheumatic heart disease. There is one main arterial stem at the base of the leaflet near the posterior commissure.
- FIG. 2. Diffusely vascularized mitral valve in a white male, 61 years of age, with no gross rheumatic heart disease. The vessels extend to the line of closure.
- FIG. 3. Vascularized anterior mitral leaflet in a white male, 65 years old, with non-deforming rheumatic heart disease, as viewed by transmitted light. There are three main arterial stems at the base of the leaflet.
- FIG. 4. Vascularized anterior mitral leaflet in a white male, 21 years old, with no gross rheumatic heart disease, as viewed by transmitted light. Two main arterial stems are present at the base of the leaflet.
- FIG. 5. Transverse section of a musculo-elastic artery, 168  $\mu$  in diameter, in an anterior mitral leaflet. The vessel wall has a honeycombed appearance. Hematoxylin and eosin stain.  $\times 415$ .



2



4



5



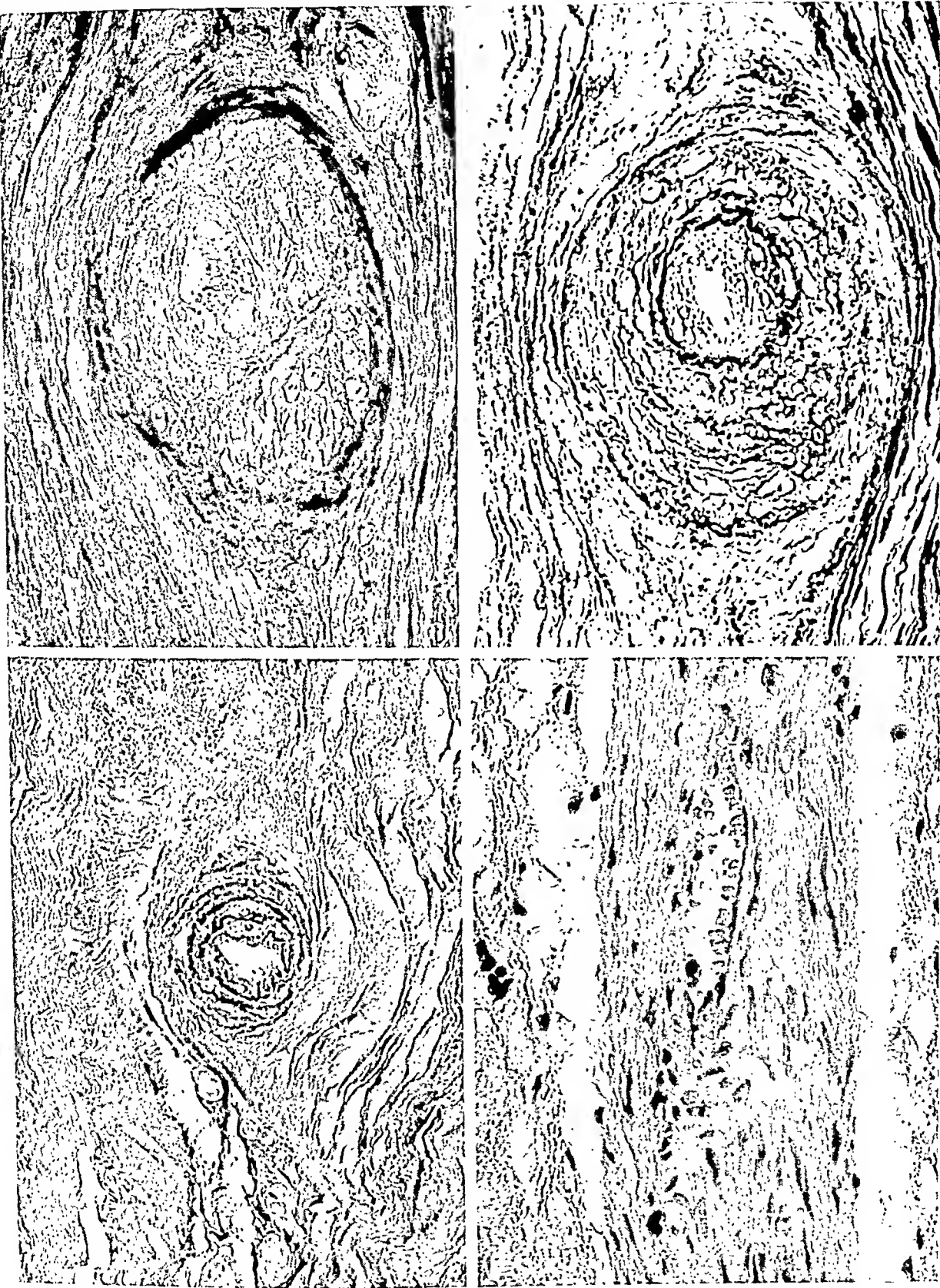
PLATE 81

FIG. 6. Same vessel as shown in Figure 5. There is a dense elastic band at the periphery of the artery. Elastica-van Gieson stain.  $\times 330$ .

FIG. 7. Transverse section of a musculo-elastic artery,  $168\ \mu$  in diameter, in an anterior mitral leaflet, showing honeycombed appearance of the wall. A prominent elastic lamina is present near the endothelium. Elastica-van Gieson stain.  $\times 330$ .

FIG. 8. Musculo-elastic vessel of arteriolar size,  $70\ \mu$  in diameter, in an anterior mitral leaflet. Elastica-van Gieson stain.  $\times 330$ .

FIG. 9. Longitudinal section of a musculo-elastic artery,  $112\ \mu$  in diameter, in an anterior mitral leaflet. The muscle fibers are arranged in the long axis of the vessel. Hematoxylin and eosin stain.  $\times 330$ .



7

9

Koletsky

Vascularity of Mitral Valve

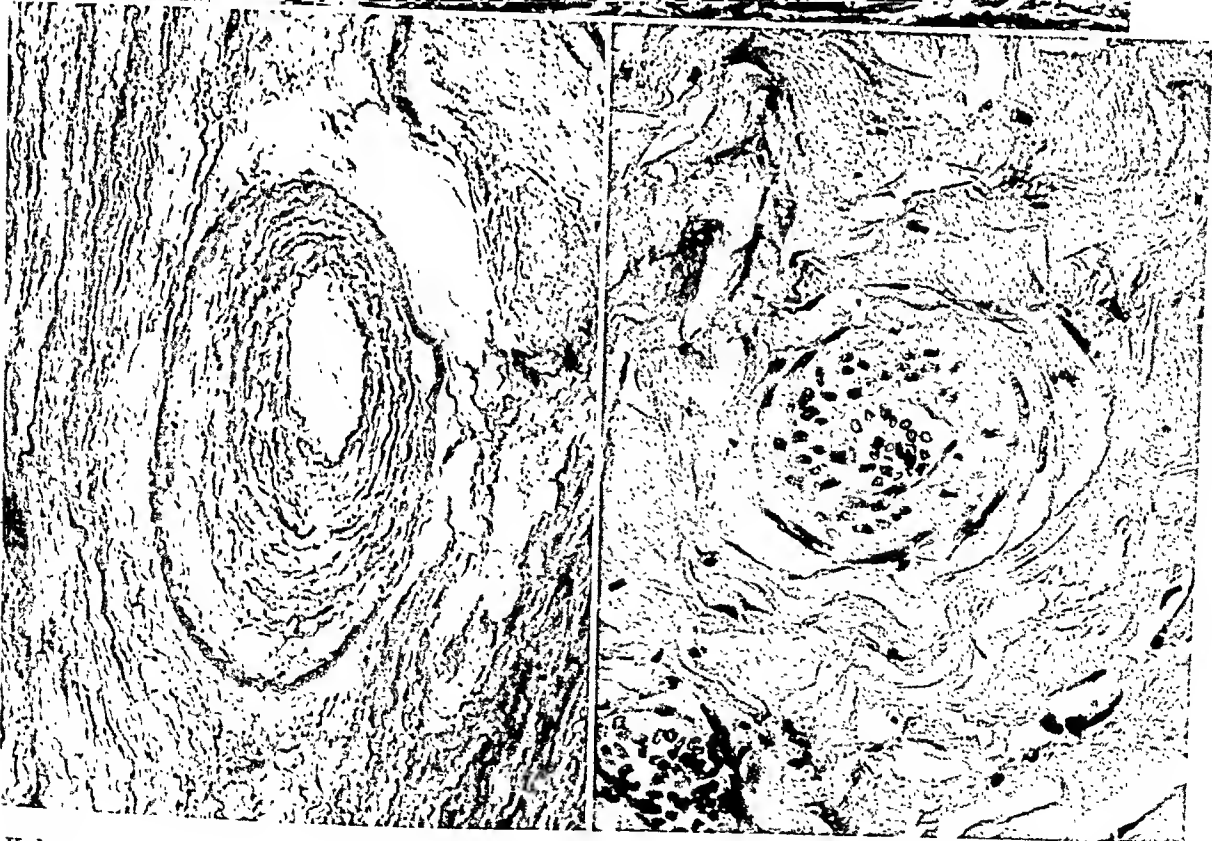
PLATE 82

FIG. 10. Artery of intimal musculo-elastic type, 90  $\mu$  in diameter, in an anterior mitral leaflet. Elastica-van Gieson stain.  $\times 415$ .

FIG. 11. Musculo-elastic artery, 140  $\mu$  in diameter, showing the fibro-elastic stage. Concentric elastic bands extend through the entire wall of the vessel. Elastica-van Gieson stain.  $\times 330$ .

FIG. 12. Artery of intimal musculo-elastic type, 100  $\mu$  in diameter, in pericardial scar. Hematoxylin and eosin stain.  $\times 330$ .

10



12

Koletsky

Vascularity of Mitral Valve



OBSERVATIONS ON THE PATHOLOGICAL CHANGES PRODUCED BY  
A TOXIC SUBSTANCE PRESENT IN BLUE-GREEN ALGAE  
(*MICROCYSTIS AERUGINOSA*)\*

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The presence of a toxic substance or substances in certain species of blue-green algae has been the subject of a number of reports.<sup>1-3</sup> In the case of one species, *Microcystis aeruginosa*, somewhat more extensive observations have been made upon the pharmacological and chemical properties of the toxic component.<sup>3,4</sup> Extracts prepared from *M. aeruginosa*, when injected subcutaneously, intraperitoneally, or intravenously, produce a characteristic response in several species of laboratory animals. A variable latent period is followed by pallor, decline in blood pressure, tachycardia, hypothermia, and death consequent to respiratory failure in a length of time dependent upon the kind of animal employed and the dose administered.<sup>4</sup> Upon autopsy, the liver is found uniformly to be dark reddish blue, and engorged, bleeding profusely after incision.

We are unaware of any detailed studies of the pathological changes produced by the toxic agent in *M. aeruginosa*. Thus it is the purpose of this communication to describe the structural alterations in such organs of rats as are affected following the administration of a maximal sublethal dose of algal extract.

#### METHOD OF STUDY

Albino rats weighing about 200 gm. were employed in all experiments. The animals were maintained on a standard colony diet. The extract employed was prepared by shaking dry algal powder (which had previously been extracted with anhydrous chloroform, acetone, and ether) with about fifteen times its weight in water for three successive times. The aqueous phases were separated by prolonged centrifugation, pooled, and transferred to a series of stoppered serological tubes and stored in the frozen state at low temperature. The toxicity of the extract thus prepared and stored appears to be maintained indefinitely. The proportions employed yielded an extract for which the maximal sublethal dose was approximately 1 cc. per 100 gm. of rat.

The extract was administered intraperitoneally to a series of rats, and these were sacrificed at intervals following the injections. Portions of liver, brain, heart, lungs, spleen, kidneys, and gastrointestinal tract

\* Received for publication, April 10, 1945.

were fixed in 10 per cent formalin. Sections were cut from paraffin and stained with hematoxylin and eosin. Sudan IV was used for staining frozen sections for fat in liver, kidney, and myocardium.

## RESULTS

### *Gross Observations*

Fifteen to 30 minutes after the injection the liver was slightly enlarged, tense, and redder than normal. The centers of the lobules showed increased blood content. There were no gross changes noted in other organs at this early stage.

The livers were markedly enlarged in animals examined 3 to 6 hours after injection of the extract, weighing about 25 per cent more than those of normal control rats. This increased weight was not accounted for by blood content of the large veins since the amount of blood which could be drained from the veins was approximately the same by weight in the poisoned animals as in the control group. There was an increased blood content in the parenchyma of the liver, however, as indicated by the bloody fluid which dripped in excess from the cut surface and by the engorgement of the central portions of the lobules. The latter feature was responsible for enlargement of the lobule as a whole. The remaining periphery of the lobules was grayish red. The liver tissue was softened and friable. In some instances the parenchyma was partially liquefied so that a thick, creamy, dark bluish red fluid could be pressed from the cut surface. There was usually a diffuse alteration so that all lobes of the liver presented the appearance described. However, in the early stages there was occasionally patchy involvement so that some lobes or portions of lobes retained an essentially normal gross structure. The hilar portions of the lobes appeared to be the regions first involved.

There was no detectable change in the weights of the heart, lungs, spleen, or kidneys in these acutely poisoned animals. The lungs were hyperemic in animals dying spontaneously but usually were normal in color and air content in animals which were sacrificed. A few rats exhibited small hemorrhages in the lung tissue. The kidneys appeared slightly hyperemic, but otherwise seemed normal.

Two to 3 days after a maximal sublethal dose, the liver had undergone remarkable changes. It was shrunk to about two-thirds its normal size and was dull yellow. It was soft and had a mottled appearance. This mottling was due to red areas marking the slightly enlarged central veins, with surrounding yellow parenchyma. The blood content of these yellow livers was not increased. The other tissues were slightly

icteric. There was no excess fluid in the serous cavities. The kidneys had a brown tinge, were slightly softened and larger than normal. The urine was dark yellow. The lungs were normal at this stage except for occasional petechial hemorrhages. The blood clotted very slowly, sometimes requiring 10 or 15 minutes. The animals had lost a great deal of weight and exhibited marked general pallor which showed, also, in the conjunctiva.

Five days after the maximal sublethal dose the liver had returned to an almost normal state. It still had a yellow tinge but the mottled appearance had disappeared and other organs were, at this time, normal in appearance. The animals still appeared cachectic, pale, and anemic. One month after the injection no gross changes in the liver or other organs could be discerned, and the animals appeared to be entirely normal.

### *Histological Observations*

*Liver.* As early as 15 minutes after intraperitoneal injection the parenchymal cells were found to be swollen and their outlines less prominent (Fig. 1). The cytoplasm had become more granular and in some areas was hydropic or pale-staining. Fat droplets (Fig. 2) appeared in cells near the center of the lobule. Sometimes there were clear halos in the perinuclear cytoplasm. At this stage the sinusoids in the centers of the lobules were already slightly distended with red blood cells. In the center of the lobules there were a few small groups of cells in which the cytoplasm was somewhat homogeneous and pink, and the nuclei were pyknotic or failed to stain, indicating small areas of coagulative necrosis.

The most advanced degree of hepatic injury was encountered in animals about 4 hours after intraperitoneal injection. There was marked necrosis involving the centers of the lobules. The extent of this necrosis varied from a few cells to almost the entire lobule. There was complete dissociation of the liver cords with remnants of liver-cell bodies lying free in the blood of the confluent sinusoids. These isolated liver cells were usually rounded and swollen. The cytoplasm was hyalinized, eosinophilic, and sometimes vacuolated as a result of fat accumulation. The fat droplets were always small, being from 4 to 8  $\mu$  in size. The nuclei of these cells were pyknotic or failed to stain. In some instances the liver cords at the center were partly preserved in form and also contained viable appearing cells, but in many of the more severely injured livers there were collections of a pale blue, homogeneous material lying in pools in the centers of the lobules where the liver cells were completely absent (Fig. 3). This blue material frequently was found to be continuous with adjacent liver cells. A few polymorphonu-



clear leukocytes and also pyknotic nuclei were present in this material. The cells remaining at the periphery were swollen, granular, slightly vacuolated with fat, and also contained some vacuoles devoid of fat.

At this relatively early stage there was but little removal of the dead cell bodies. The sinusoids at the center were markedly distended, apparently confluent, and filled with red blood cells (Fig. 4). There was actual destruction of the sinusoidal endothelium in some instances and the sinusoidal spaces had become confluent, leading to large lakes of blood.

At 24 to 48 hours the number of dead liver cells was markedly diminished and the architecture of the lobule fairly well restored. The Kupffer and endothelial cells at the center of the lobule were present and apparently formed continuous sinusoids. The parenchymal cells were still swollen, granular, and contained many fine fat droplets. The amount of blood in the sinusoids was very greatly reduced compared to the earlier stages.

At 3 to 5 days after an almost lethal dose of the extract, the liver lobules were normal in size and arrangement. Rare coagulative, rounded cell bodies remained around the central vein. The liver cords were intact. The sinusoids contained a normal amount of blood. The liver cells were swollen with somewhat granular cytoplasm, and had enlarged, hyperchromatic nuclei. Mitotic figures were commonly seen in the parenchymal cells, indicating regeneration (Fig. 5).

After 28 days the liver was completely restored to normal as far as histological study could disclose.

*Heart.* As early as 2 hours after administration of the extract there were swelling and increased granularity of the muscle fibers. Scattered, hyalinized and slightly swollen muscle fibers were present in the left ventricle. Underneath the endocardium and in the myocardium were a few small, recent, interstitial hemorrhages and a more diffuse acute hyperemia.

These changes were most marked at 4 hours, diminishing in intensity thereafter, until at 3 to 5 days there were no demonstrable changes.

*Lungs.* The lungs were usually only slightly involved. The presence of acute hyperemia was variable. In some instances the alveolar capillaries were actually smaller, in others they were definitely distended. The most common finding was focal, slight, recent alveolar hemorrhage. In a few animals both alveolar and interstitial edema were present.

*Kidney.* The earliest alteration in the histological appearance of the kidneys was noted 20 minutes after injection. This consisted of a marked, patchy, cortical and medullary hyperemia (Fig. 6). Somewhat later there were swelling, increased granularity, and loss of dis-

tinged luminal border of the convoluted-tubular epithelial cells. In some instances there was necrosis involving only small groups of cells and segments of convoluted tubules. Hydropic swelling and apparent cell disintegration of some of these necrotic cells were observed. A constant finding was the presence of a blue, almost homogenous but finely granular, often loose or reticulated deposit within the lumina of the tubules.

In some rats the cytoplasm of degenerated convoluted tubular epithelium contained small brownish granules resembling bile pigment. Occasionally, hyaline droplets were noted in the cytoplasm.

Sections of spleen, suprarenal glands, intestine, and brain revealed no changes.

### DISCUSSION

As indicated by these observations, the toxic substance of *M. aeruginosa* is a rapidly acting cytotoxic agent with particular tendency to damage hepatic parenchymal cells. Its action, however, is not confined to the liver, but leads also to cellular degeneration and necrosis in a much less intense form in other organs, particularly the heart and kidneys. The early occurrence of parenchymatous degeneration of these organs before actual necrosis is present in the liver seems to indicate that the muscle fibers of the heart and the epithelium of the kidney are injured directly by the poison rather than as a result of the hepatic necrosis which follows. The development of petechial hemorrhages in the heart and lungs might indicate that this agent also injures capillary endothelial cells, and the effect of this may be exaggerated by the prolonged clotting time of the blood. The same mechanism might conceivably account for the hyperemia of the various organs, but circulatory failure would seem to be a better explanation.

The first stage of hepatic injury can be considered as the pre-necrotic stage in which progressive degenerative changes in the parenchymal cells are in evidence. The extreme rapidity of action of the toxic substance is indicated by advanced parenchymal degeneration as early as 15 minutes after injection. This rapid response suggests that the poison acts directly upon liver cells without any intermediate changes in the body. The first structural change brought about in the liver cells is an increase in their size, apparently due to increased fluid content. This is accompanied by increased granularity of cytoplasmic substance and, shortly afterwards, by the appearance of fat droplets and fat-free vacuoles within the cytoplasm. The immediate explanation for these alterations is not readily apparent, but it is possible that, as a result of the direct action of the toxin upon the cells, there is increased osmotic pressure within the cells leading to intracellular edema, and that

metabolic processes are altered in such a way that unutilized fat collects in the cytoplasm.

The second stage of injury in the liver is that of necrosis and engorgement. The necrosis begins in the center of the lobule, perhaps because of poorer oxygen supply in this region. The steps leading to necrosis of parenchymal cells can be readily traced from acute parenchymatous to fatty degeneration, and, with or without the intervention of hydropic change, to coagulative necrosis. A remarkable feature is the rapid liquefaction of dead cells. Within 4 hours there is advanced disruption of the cell cords, indicating that many dead cells have been removed. Very striking is the stage intermediate between coagulative necrosis and cytolysis, in which masses of blue-staining cytoplasm lie free in the sinusoids, the cell membranes having apparently disintegrated. This liberation of cytoplasm into the sinusoidal spaces probably leads to a rapid removal of liver parenchymal substance by solution in, or disintegration into, the blood within the lobule. The possible consequences of this liberation of dead liver-cell cytoplasm into the blood stream have not yet been assessed. Associated with necrosis is a marked accumulation of blood within the center of the lobule. This collection of blood is considered to be located largely within markedly widened or hyperemic sinusoids, but in some instances sinusoidal walls have apparently disintegrated with hemorrhage occurring within the lobule. Collection of blood in the centers of lobules is not entirely dependent upon widening of sinusoids from disappearance and thinning of liver cords, for the lobule as a whole is increased in size. The blood which collects in this manner may be of sufficient volume to be an important factor in the circulatory failure which occurs terminally in the acutely poisoned animals.

The next stage of hepatic injury is that in which there is effective removal of dead cells. This appears to proceed rapidly, having begun as indicated about 4 hours after administration of the extract. About 24 hours following administration the lobule is collapsed and smaller. Most of the dead cell bodies have apparently been removed by autolysis or by the cytoplasm being carried away in the sinusoidal blood. At this time, however, there is still some persistence of the parenchymal damage as shown by acute parenchymatous and fatty degeneration in the liver cells which remain. Following removal of dead liver cells, the period of regeneration becomes evident at 3 to 5 days. Regeneration apparently proceeds within the lobule, the general architecture of which remains intact. Regeneration is indicated by restoration of the cell cords, by increased numbers of sinusoidal endothelial cells, by hypertrophy of parenchymal cells, and by mitotic figures. The formation of

new parenchymal cells takes place from pre-existing liver cells. Bile duct regeneration plays no part in this process, nor does it appear to occur here in any form. The process of regeneration and restoration proceeds so that at 28 to 30 days there is no evidence of the previous hepatic injury. The lobules are completely reconstituted, being normal in size and architecture. There is no evidence of nodular regeneration or fibrosis.

This completeness of restoration would seem to be of significance in connection with the problem of hepatic cirrhosis developing from severe injury. With injury of the type produced in these experiments, which is intense with widespread necrosis, it appears that a single episode without persistence of the damaging agent does not lead to cirrhosis, but, instead, to complete restoration of the liver substance. This observation is in accord with that of Lucké<sup>5</sup> who found complete return to normal structure in the livers of patients who had had acute epidemic hepatitis with necrosis of the liver, and also agrees with many studies regarding acute hepatic injury produced by such agents as chloroform and carbon tetrachloride.

The matter of renal injury deserves special comment. The degenerative changes occurring in the convoluted tubules suggest the excretion of substances capable of damaging the tubular epithelium. Bile pigment and bile salts are almost certainly eliminated in increased amounts in the urine of these rats, and in a few the renal epithelium contained granules of bile pigment. However, bile-stained casts and abundant bile pigment in the epithelium were usually missing; and therefore probably did not account for the tubular changes. A second possible cause is the excretion of cytoplasmic products from liquefied liver cells. The blue-staining granular deposit in the tubules suggested such material, but it was not positively identified. Another possible cause of the renal injury is the toxic substance itself, as previously suggested. The fate of the poison within the body, with possible elucidation of the renal injury, is to be studied.

The pathological findings afford some suggestions as to the mechanism of death. There seem to be four separate groups of changes, which individually or collectively might be concerned. First, the necrosis of the liver is so extensive that acute hepatic insufficiency might cause death. Rats dying 24 hours or more after the injection exhibit signs suggestive of hepatic coma. Moderate to profound lowering of the glucose of the blood has been observed at this stage. However, it is improbable that deaths occurring only a few hours after the injection are due to liver insufficiency in this sense. The development of a state of shock in animals dying early, with decreased blood pres-

sure and hypothermia, may account for death. The mechanism of the shock associated with this poisoning has not been completely elucidated, but two probable factors are the collection of blood in the liver and the associated vasodilatation and/or hyperemia of other organs. Another condition which might account for some delayed deaths is that of renal insufficiency consequent to acute tubular degeneration. Finally there is possibly a depression of cellular metabolism in all organs of the body as indicated by parenchymal degeneration, although no such effect could be demonstrated in an earlier study in terms of oxygen consumption of isolated tissues to which extract containing the toxic substance was added, according to the Barcroft-Warburg technic.<sup>4</sup> These observations on the mechanism of death are provisional, being dependent upon deductions from structural changes.

The hepatic injury produced by the poison of *M. aeruginosa* is distinguished from that brought about by a variety of hepatotoxic agents chiefly by the remarkable rapidity of the development of the lesions, the quick disintegration of dead liver cells, and the intense engorgement of the liver with blood. Necrosis, chiefly of the center of the lobules, and the associated parenchymal degeneration and regeneration of the lobules in recovery are observed following administration of chloroform,<sup>6</sup> phosphorus,<sup>7</sup> and numerous other substances<sup>7-9</sup> and in epidemic hepatitis. A considerable similarity exists between this algal poison and the thermostable amanita toxin of the mushroom, *Amanita phalloides*,<sup>10-12</sup> in respect to pathological effects, chemical properties, and composition.<sup>13</sup> Marked increase of tolerance to algal extract after repeated administration is observed just as with amanita-toxin, and other evidence indicates that the active substances may be similar, if not identical.<sup>13</sup>

Various observations in connection with chronic administration of algal poison will be reported subsequently. -

#### SUMMARY

1. The toxic substance which can be extracted from *Microcystis aeruginosa* leads, upon administration to rats, to generalized cellular damage with particularly severe injury to the parenchymal cells of the liver.
2. The injury in the liver can be followed through successive stages of acute parenchymatous, hydropic, and fatty degeneration, to necrosis in the centers of the lobule. The dead liver cells disintegrate rapidly by cytolysis and by fragmentation of cytoplasm into the circulating blood. There is marked engorgement of the centers of the lobules.

3. The dead liver cells are removed at 24 to 48 hours, regeneration is in progress at 3 to 5 days, and there is complete restoration of liver lobules within 30 days after administration of the extract.

4. There is no evidence of nodular regeneration or fibrosis from a single severe injury produced by this hepatotoxic agent.

5. Acute parenchymatous and hydropic degeneration, and focal necrosis as well as hyperemia are observed in the heart and kidneys. Small hemorrhages and occasionally edema occur in the lungs.

6. Early death from acute poisoning can probably be attributed to shock and circulatory collapse. Delayed death is probably consequent to hepatic insufficiency, renal failure, and generalized cellular damage by the toxic agent.

7. The types of structural alterations in the liver produced by the agent are similar in many respects to those produced by several known hepatotoxic substances.

The photomicrographs were prepared by Mr. Lewis Waters of the Medical Arts Department of the Southwestern Medical School.

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## DESCRIPTION OF PLATES

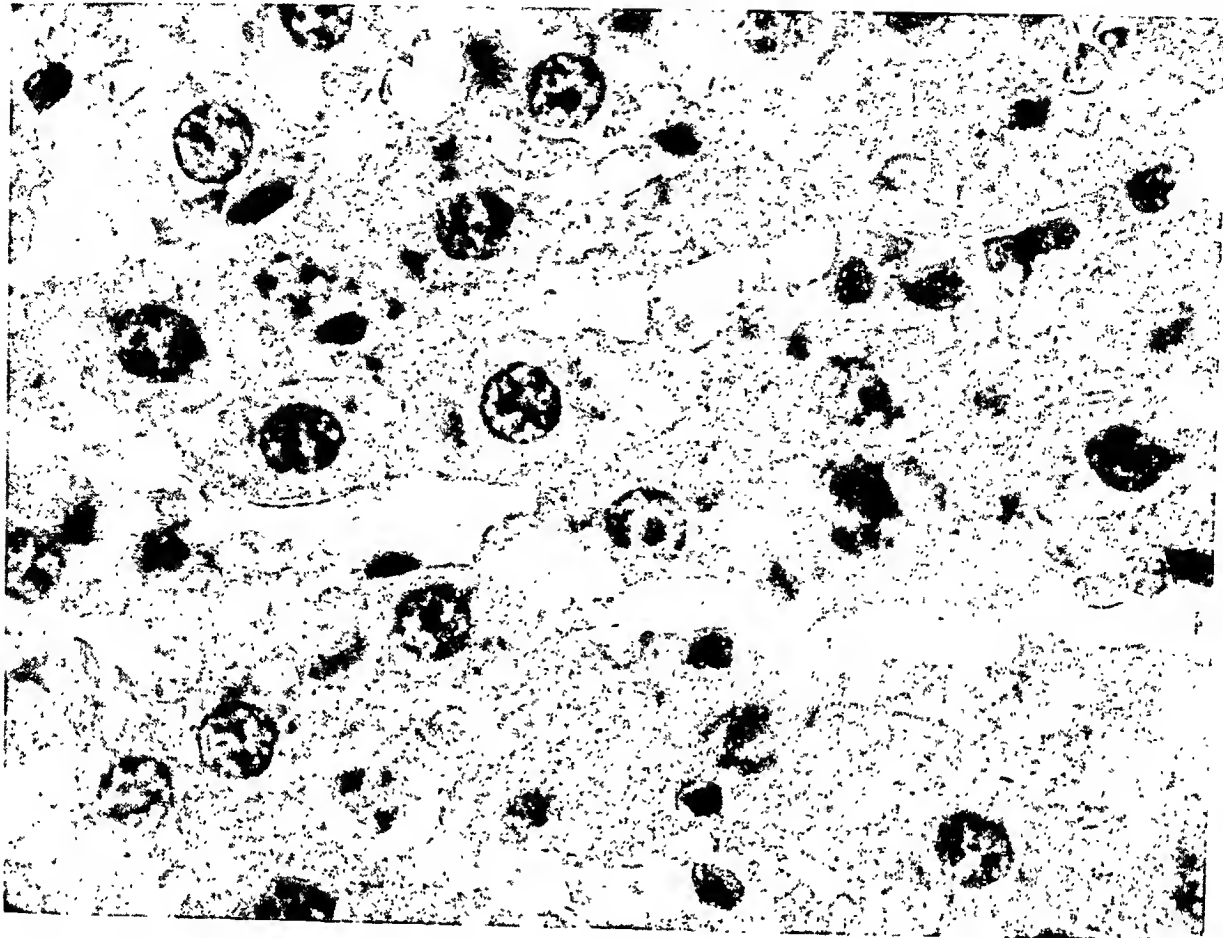
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### PLATE 83

FIG. 1. Early parenchymatous degeneration of liver, 15 minutes after injection of algal extract. Hematoxylin and eosin stain.  $\times 1170$ .

FIG. 2. Fatty degeneration at center of lobule, 30 minutes after injection. Hematoxylin and eosin stain.  $\times 380$ .

1



2

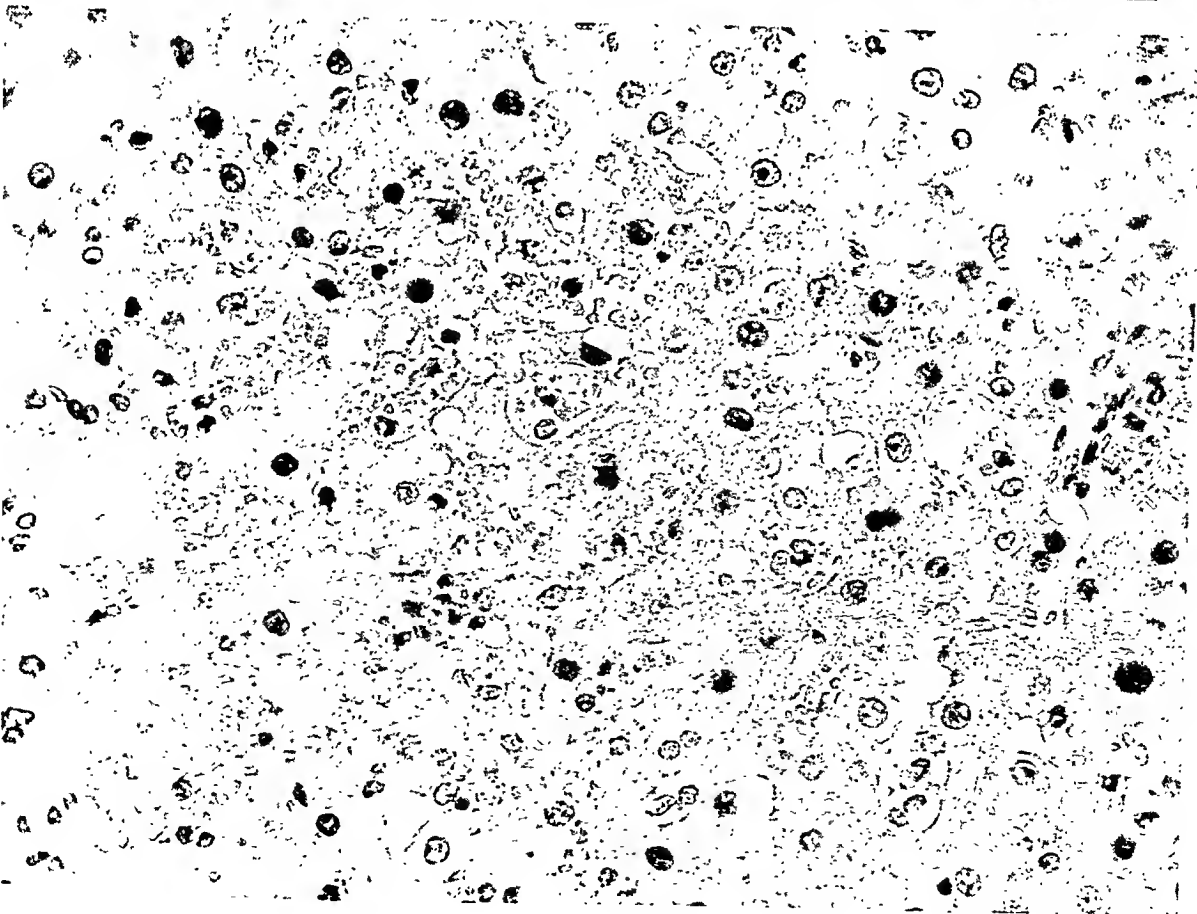


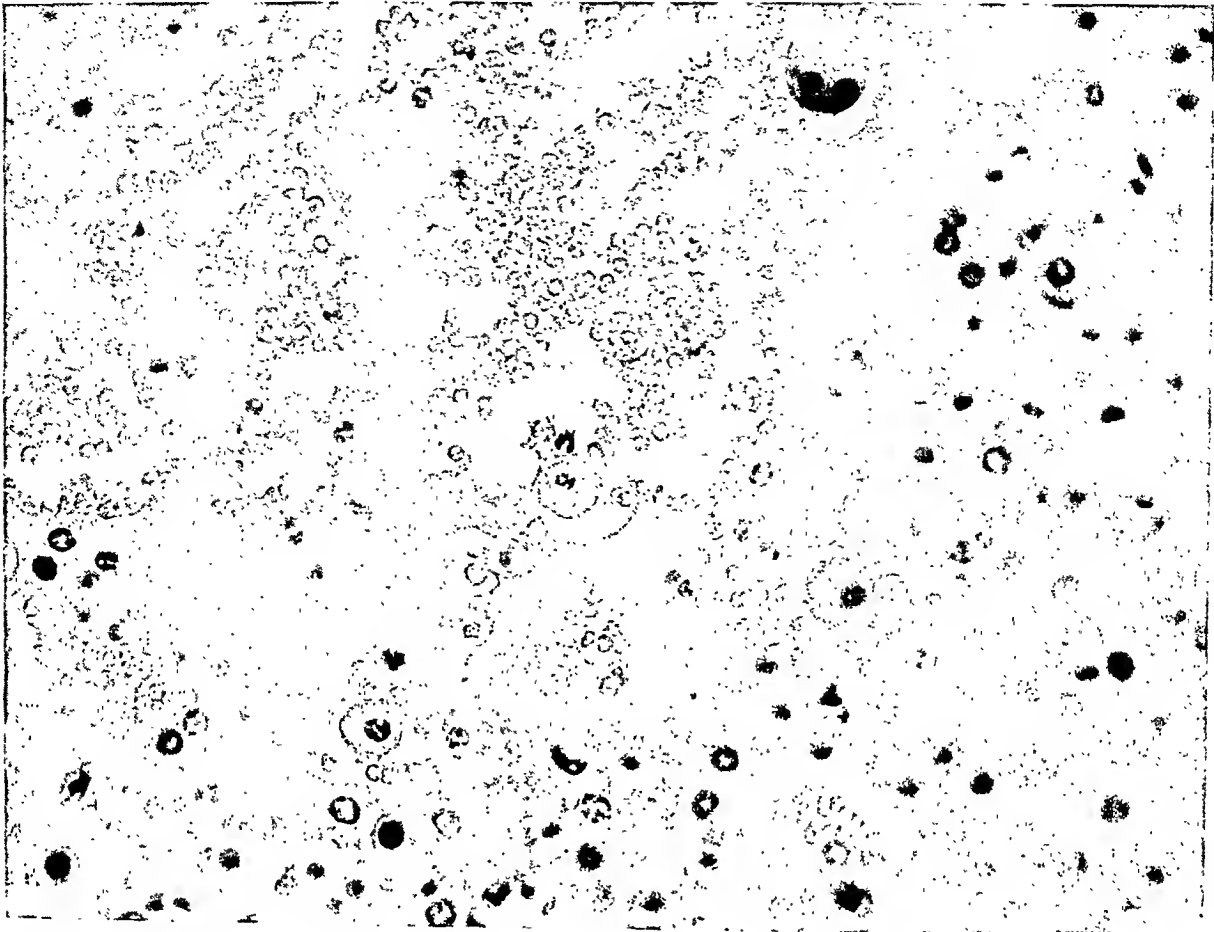


PLATE 84

FIG. 3. Early stage of central necrosis, with cytoplasmic material lying free in sinusoids. Hematoxylin and eosin stain.  $\times 380$ .

FIG. 4. Marked accumulation of red blood cells in sinusoids in necrotic portion of liver lobules. Hematoxylin and eosin stain.  $\times 380$ .

3



4

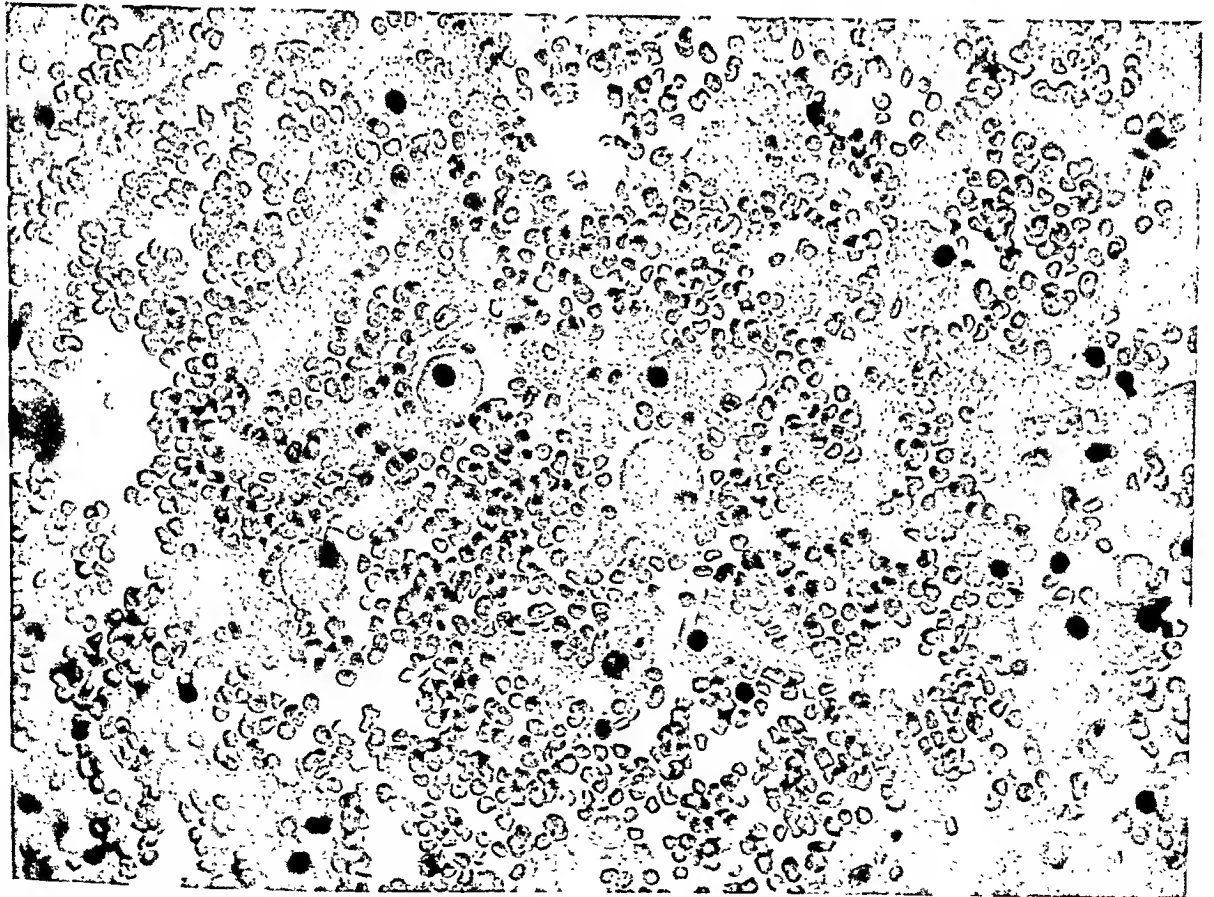
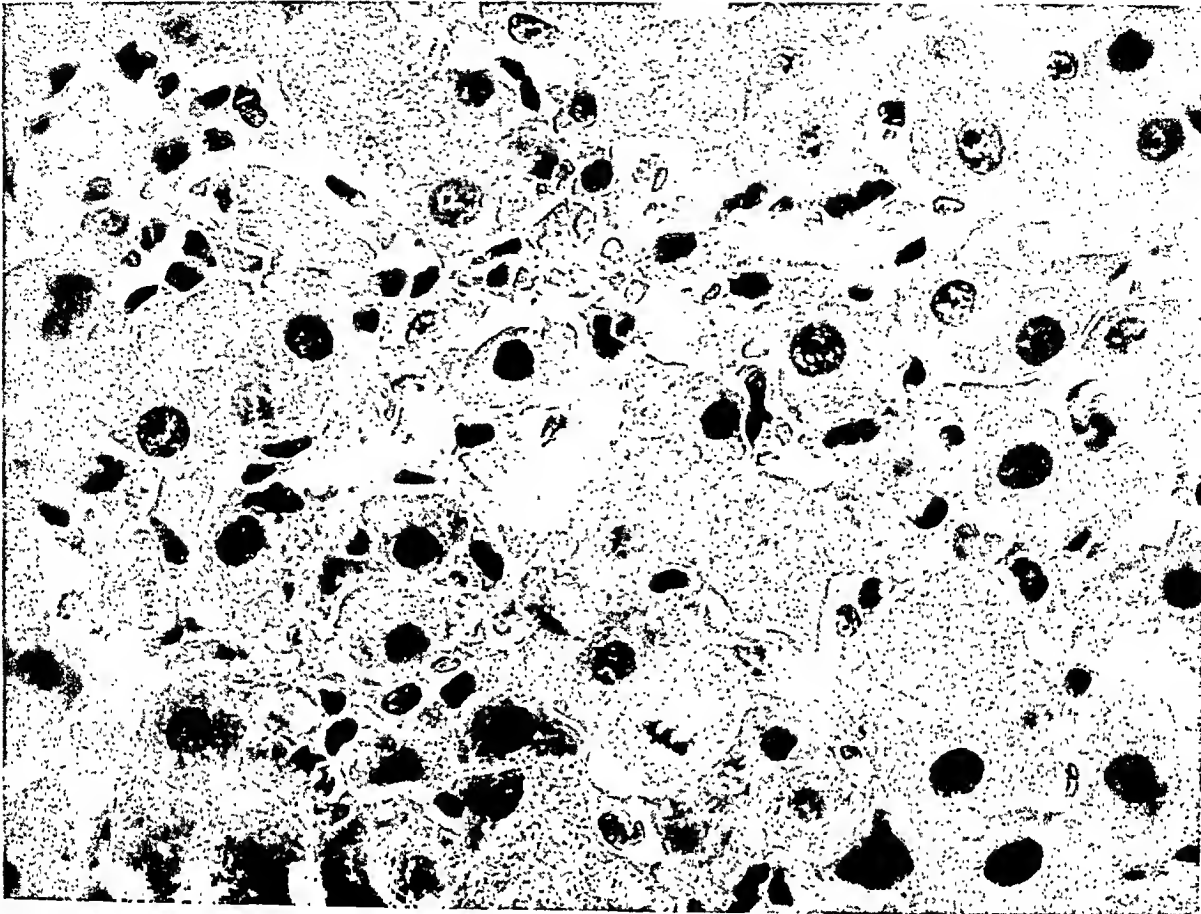


PLATE 85

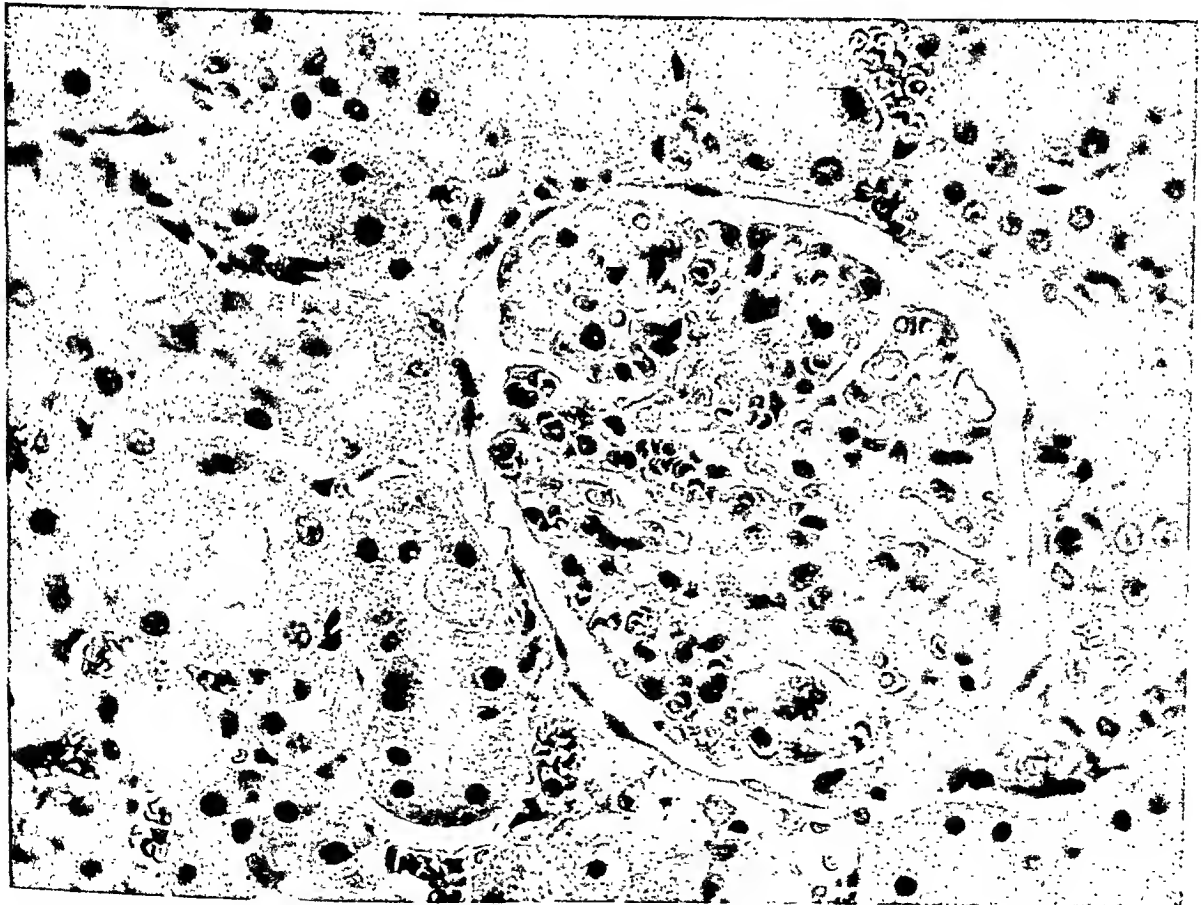
FIG. 5. Regeneration of liver cells, 5 days after sublethal injection of algal extract. Hematoxylin and eosin stain.  $\times 585$ .

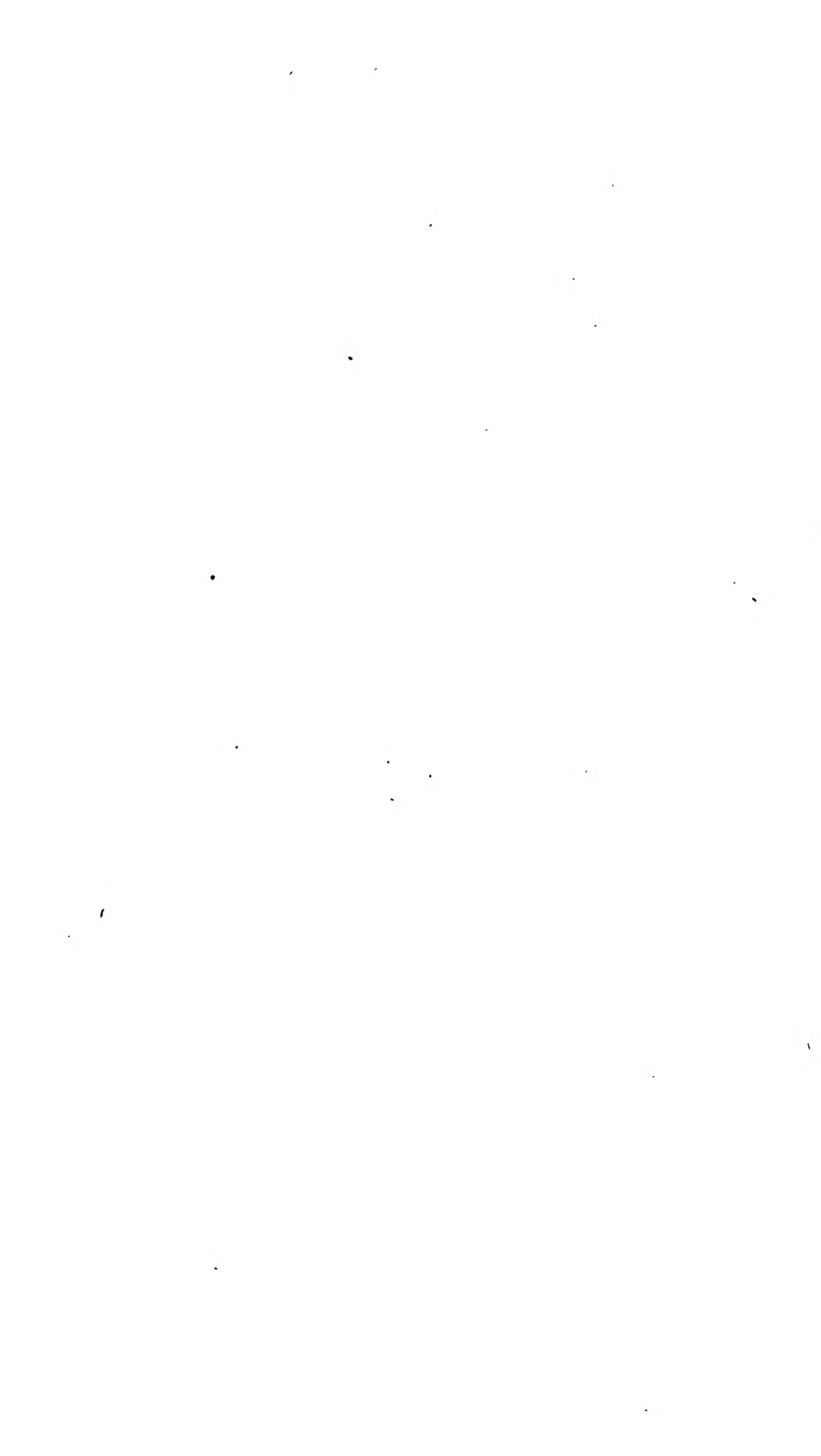
FIG. 6. Acute passive hyperemia and parenchymatous degeneration in kidney 5 hours after administration of the extract. Hematoxylin and eosin stain.  $\times 380$ .

5



6





## THE NEPHROTOXIC ACTION OF *dl*-SERINE AS RELATED TO CERTAIN DIETARY FACTORS \*

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Renal necrosis in rats receiving *dl*-serine by stomach tube has been described in a previous report.<sup>1</sup> In those experiments one group of animals was maintained on a stock diet which was considered adequate, while another received a synthetic diet known to be deficient in the B vitamins and low in protein. Within a few days after the administration of *dl*-serine was begun, both groups of rats developed renal necrosis. Although the amino acid was still supplied daily, the animals maintained on the stock diet showed rapid and almost complete repair of the kidney tubules, while those on the deficient diet developed progressive necrotic lesions which were associated with calcification. In the latter group regenerative processes were also apparent, but the injury was so extensive that restoration of the renal parenchyma to its previous state was impossible.

Among the other findings noted was increased capillary permeability, progressing in certain instances to hemorrhagic transudation; these vascular changes were much more pronounced in the animals maintained on the deficient diet. Since the fatalities which occurred were confined to this group, peripheral circulatory failure was suggested as a possible mechanism of death.

The pathological findings after serine administration confirmed the results of previous experiments in which the clinical symptoms and the mortality were studied.<sup>2,3</sup> In an effort to explain the less injurious action of serine upon rats on the stock diet, possible deficiencies of the experimental diet were taken into consideration. It was felt that the diet was deficient in B vitamins, choline, cystine, and glycine.

A later study showed that supplementing the experimental diet with a mixture of pure B vitamins alleviated the severity of clinical symptoms and considerably reduced the mortality in rats receiving serine by stomach tube.<sup>4</sup> Of the individual B vitamins tested, pyridoxine seemed to be most effective. The best results, however, were obtained in rats maintained on the experimental diet supplemented with choline, cystine, glycine, and the complete mixture of B vitamins.

In the present study similar experiments have been performed with

\* Aided in part by a grant from the John and Mary R. Markle Foundation.  
Received for publication, April 11, 1945.

the object of determining whether the pathological changes induced by serine administration are also influenced favorably by these various dietary factors.

### MATERIAL AND METHODS

Male albino rats of the Rockland strain, weighing approximately 100 gm. each, were divided into four groups and placed on an experimental diet (diet 4), the composition of which has been indicated previously.<sup>1</sup> The animals in group 1 received a supplement of 50  $\mu$ g. of pyridoxine daily. For the rats in group 2 the supplement consisted of a mixture of B vitamins, containing 50  $\mu$ g. of thiamine hydrochloride, 50  $\mu$ g. of riboflavin, 1,500  $\mu$ g. of nicotinic acid, 200  $\mu$ g. of calcium pantothenate, 200  $\mu$ g. of inositol, and 200  $\mu$ g. of p-aminobenzoic acid daily. The animals in group 3, in addition to these B vitamins, received also 50  $\mu$ g. of pyridoxine daily. Rats in group 4 received the same complete B vitamin mixture as those in group 3, and, in addition, were given daily supplements of choline hydrochloride, *l*-cystine, and glycine (50 mg. each). The vitamins were administered parenterally, whereas choline, cystine, and glycine were incorporated into the diet. As far as possible, the food was supplied to the animals in amounts slightly in excess of their requirements. Food consumption was checked daily, and the rats were weighed every third day. Water was given *ad libitum*.

After the animals of all groups had been maintained on the experimental diets for a period of 7 days, they received by stomach tube 100 mg. of *dl*-serine, dissolved in 3 cc. of water, given daily for a maximum of 14 days. A small number of rats in each group were designated as controls and did not receive the amino acid.

Surviving animals of all groups were sacrificed by decapitation at various intervals (Tables I to IV). Representative tissues from each rat were placed in a 4 per cent solution of neutral formaldehyde. The pieces of tissue were embedded in paraffin, cut at 6  $\mu$ , and stained with hematoxylin and eosin.

### RESULTS

Examination of the accompanying tables reveals that none of the dietary substances tested were capable of preventing the renal and vascular changes which ordinarily follow the administration of *dl*-serine to animals on the experimental diet (diet 4). The renal lesions were those of prompt necrosis and extensive calcification in the tubules (Figs. 1 to 4), associated with proliferation of fibroblasts, tubular dilatation with regeneration of epithelium, and mononuclear, eosinophilic, and neutrophilic cellular infiltration. In 5 of the 11 animals employed as controls, the kidneys showed calcium deposits in the form

of flecks and sheets, but these deposits in most instances were minimal in degree (Fig. 5).\*

The vascular changes following serine administration consisted of general capillary dilatation, presumably associated with increased permeability. Red blood cells were present in the pulmonary alveoli of most of the animals and varied in amount from minute hemorrhagic foci scattered throughout the lung substance to almost complete hemorrhagic consolidation (Fig. 6). None of the substances tested appeared

TABLE I  
*Experimental Diet + Pyridoxine + Serine*

No. of animal	Duration of experiment	Days receiving serine	Degenerative kidney disease	Hemorrhagic transudation (lungs)	Myocarditis	Fatty liver
	(days)					
A-34-3	9	1	Calcification	+	—	+
A-34-4	9	1	Necrosis, calcification	++	+	—
A-33-5	13	5	Necrosis, tubular dilatation and regeneration, calcification	++	+	+
A-33-6	13	5	Necrosis, tubular dilatation and regeneration, calcification	++	+	+
A-33-1	22	14	Tubular dilatation and regeneration, calcification	++	—	—
A-33-2	22	14	Tubular dilatation and regeneration, calcification	—	—	—
A-33-3	29	14	Tubular dilatation and regeneration, calcification	++++	—	+
A-33-4	29	14	Normal kidneys	+++	+	—
<i>Controls</i>						
A-34-5	8	0	Calcification	—	+	—
A-34-6	8	0	—	—	+	—
A-31-5	21	0	—	—	+	+
A-34-1	28	0	—	—	+	+
A-34-2	28	0	—	—	—	+

to affect the process. In the control animals, however, vascular changes were either absent or minimal in degree.

Myocarditis was present in approximately 50 per cent of the animals, and occurred in all groups. The lowest incidence, however, was found in the group of animals receiving the complete B-vitamin supplement (group 3). The rats of group 4, on the other hand, showed a high incidence of myocardial disease, although they had received supplements of choline hydrochloride, cystine, and glycine in addition to the complete mixture of B vitamins. The disease was characterized by a degeneration of the myocardial fibers and mononuclear infiltration (Fig.

\* The changes in the kidneys of the animals employed in this study were identical with those described in a previous report<sup>1</sup> and therefore have not been illustrated in detail here.



7). Eosinophils and neutrophils were seen in small numbers, and there was some fibroblastic activity. The process was most marked in the auricles.

TABLE II  
*Experimental Diet + Complete B-Vitamins Mixture — Pyridoxine + Serine*

No. of animal	Duration of experiment (days)	Days receiving serine	Degenerative kidney disease	Hemorrhagic transudation (lungs)	Myocarditis	Fatty liver
A-31-1	9	1	Necrosis	—	+	—
A-31-2	9	1	Necrosis	++	—	+
A-32-5	13	5	Necrosis, tubular dilatation and regeneration, calcification	+	—	—
A-32-6	13	5	Necrosis, tubular dilatation and regeneration, calcification	++	+	+
A-32-1	22	14	Tubular dilatation and regeneration, calcification	+++	+	+
A-32-2	22	14	Tubular dilatation and regeneration, calcification	++	+	+
A-32-3	29	14	Calcification	Multiple abscesses	+	+
A-32-4	29	14	Normal kidneys	—	—	+
<i>Controls</i>						
A-31-3	8	0	—	—	+	+
A-31-4	8	0	—	++	+	—

With the exception of those rats whose diets were supplemented with choline (group 4), the majority of the animals which received serine developed fatty changes in the liver. These changes consisted in a gradual increase of hepatic fat which later was associated with degenerative changes in the liver.

TABLE III  
*Experimental Diet + Complete B-Vitamins Mixture + Serine*

No. of animal	Duration of experiment (days)	Days receiving serine	Degenerative kidney disease	Hemorrhagic transudation (lungs)	Myocarditis	Fatty liver
A-36-1	9	1	Necrosis, calcification	—	—	—
A-36-2	9	1	Necrosis, calcification	++	—	—
A-39-1	13	5	Necrosis, tubular dilatation and regeneration	+++	—	+
A-39-2	13	5	Necrosis, tubular dilatation and regeneration, calcification	—	—	—
A-37-4	22	14	Tubular dilatation and regeneration, calcification	++	—	+
A-37-6	22	14	Tubular dilatation and regeneration, calcification	—	+	+
<i>Controls</i>						
A-36-3	8	0	Calcification	++	—	+
A-36-4	8	0	Calcification	—	—	—
A-37-1	21	0	Calcification	—	—	+
A-37-2	21	0	Calcification	—	+	+

The findings with respect to the clinical appearance of the rats, changes in weight, and food consumption in the present experiments closely resembled those previously described.<sup>4</sup> Since animals were sacrificed at intervals, no statement can be made regarding the mortality in the various groups.

### DISCUSSION

It was clear from our previous experiments<sup>1, 2</sup> that both clinical symptoms and pathological alterations induced by serine were less marked in animals on the stock diet than in those on the experimental diet. Among the possible factors which may favor the nephrotoxic action of *dl*-serine, the low protein level of the experimental diet (diet 4) was considered. In earlier experiments, however, the casein content of

TABLE IV  
*Experimental Diet + Complete B-Vitamins Mixture + Choline + Cystine + Glycine + Serine*

No. of animal	Duration of experiment	Days receiving serine	Degenerative kidney disease	Hemorrhagic transudation (lungs)	Myocarditis	Fatty liver
	(days)					
A-41-1	8	1	Necrosis	+++	—	—
A-41-5	8	1	Necrosis	++++	+	—
A-40-5	13	5	Normal kidneys	++	—	—
A-40-6	13	5	Necrosis, tubular dilatation and regeneration, calcification	++	+	—
A-41-3	14	6	Tubular dilatation and regeneration, calcification	+++	+	—
A-40-1	15	7	Tubular dilatation and regeneration, calcification	—	—	—

the experimental diet had been increased from 10 per cent (diet 4) to 30 per cent (diet 2).<sup>2</sup> Prior to the administration of serine, this diet provided a very satisfactory rate of growth, almost identical with that of animals on a stock diet considered adequate. However, when serine was administered, the additional amount of casein apparently made no difference in the severity of the clinical symptoms or in the mortality. The autopsy findings in the animals on this diet which succumbed during the experiments (unpublished data) did not differ qualitatively or quantitatively from those in animals which had received only 10 per cent casein in their diets. It was therefore suggested that the protective action of the stock diet against the toxic effect of serine was not due merely to the maintenance of a state of good nutrition but rather to some specific factor(s) in the diet. The experimental diet (diet 4) was almost certainly deficient in choline, cystine, glycine, and B vitamins. It appeared reasonable to determine whether or not one or more of

these substances could modify the severity of the pathological changes induced by serine.

In the experiments which constitute the basis of this report, white male rats weighing approximately 100 gm. were divided into four groups and placed on diet 4 for the duration of the experiment. The vitamins and other substances mentioned were administered as outlined in the tables. During the course of the administration of serine, animals in all four groups developed pronounced changes in their kidneys similar to those which have been described in animals maintained on an experimental diet deficient in the substances mentioned above. In none of the groups did the supplementary substance or substances administered appear to alter in any way the nephrotoxic action of the amino acid.

This finding is in contrast to the definite beneficial effect of the B vitamins, especially pyridoxine, as judged by changes in body weight, food consumption, clinical appearance, and mortality.<sup>4</sup> Apparently this beneficial action is exerted by means of an extra-renal mechanism.

On the other hand, since the renal damage caused by serine is actually less severe in the animals on the stock diet, one may postulate the existence of some factor in this diet (other than those tested) directly protecting the kidney.

Areas of calcium deposit were present in the kidneys of certain of the control animals. This was to be expected, however; for it has been pointed out that rats on the experimental diet alone (diet 4) develop small calcium deposits in their kidneys.<sup>1</sup> Renal calcification has been noted under a variety of experimental conditions,<sup>5</sup> and Hummel and Barnes<sup>6</sup> have described calcium deposits in the kidneys of rats maintained on a basal diet adequate in all respects except for caloric supply.

In the present studies no significant differences in the vascular changes were observed among the various groups of animals. It must be remembered, however, that the material consisted only of surviving animals. If the mechanism of death following the administration of serine is peripheral circulatory failure, then the most severe cases would not have been included. On this basis the mortality figures should reflect the severity of the vascular lesions. Accordingly, the hypothesis cannot be excluded that this may be the extra-renal mechanism through which the B vitamins may exert their protective action.

Myocarditis was present in all groups and therefore cannot be considered the result of serine administration. In other studies<sup>1</sup> it was noted among all groups of animals, including those on a stock diet which did not receive serine and which were sacrificed as controls. The higher incidence of myocarditis among the rats of groups 1 and 2 may

suggest, however, a relationship between the diet and the incidence of myocarditis in rats. In this connection, Ashburn and Lowry<sup>7</sup> have described a high incidence of myocarditis in thiamin-deficient rats.

### SUMMARY AND CONCLUSIONS

The administration of ample amounts of B vitamins to rats maintained on an experimental diet did not alter quantitatively or qualitatively the toxic action of *dl*-serine on renal tissue. Identical findings were obtained in rats on the experimental diet supplemented with choline hydrochloride, cystine, and glycine, in addition to the complete B-vitamins mixture. Increased capillary permeability followed the administration of *dl*-serine in all groups of rats. These findings are discussed in connection with those of previous experiments in which these substances were found to alleviate the clinical symptoms of serine injury.

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[ Illustrations follow ]

## DESCRIPTION OF PLATE

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### PLATE 86

FIG. 1. A section of the kidney removed from an animal belonging to group 1. This animal was sacrificed after having received *dl*-serine on 5 successive days. A destructive lesion is seen at the junction of the cortex and medulla, as well as extensive calcium deposits and dilated tubules.  $\times 8$ .

FIG. 2. Similar lesion from a kidney of an animal belonging to group 2.  $\times 8$ .

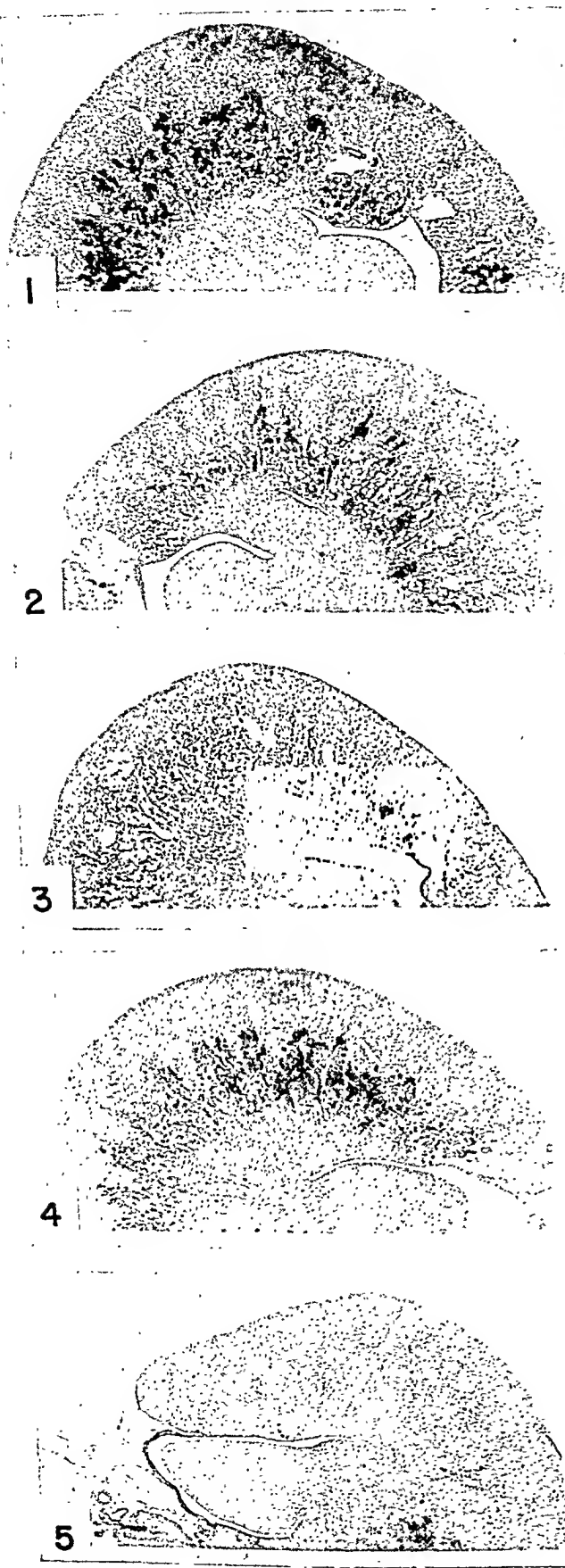
FIG. 3. Similar lesion from a kidney of an animal belonging to group 3.  $\times 8$ .

FIG. 4. Similar lesion from a kidney of an animal belonging to group 4.  $\times 8$ .

FIG. 5. Section of kidney removed from an animal employed as a control for group 3 and sacrificed after having been on the experimental diet for 21 days. No morphological changes can be seen in this section.  $\times 8$ .

FIG. 6. Hemorrhagic pulmonary transudation. The bronchioles as well as some of the alveoli are filled with blood.  $\times 110$ .

FIG. 7. Myocarditis. There is mononuclear infiltration and myocardial degeneration of the auricle.  $\times 110$ .



Morehead, Fishman, and Artom

Nephrotoxic Action of dl-Serine



## "CEROID" PIGMENT IN HUMAN TISSUES \*

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The term "ceroid" was first applied by Lillie, Daft, and Sebrell<sup>1</sup> to a coarsely globular, yellow, wax-like pigment found in the cirrhotic livers of rats maintained on a low protein, low fat diet. The pigment is characterized by its insolubility in lipid solvents, its affinity for fat stains and for basic aniline dyes, methyl green in particular, its acid-fastness when stained with the Ziehl-Neelsen method, even after prolonged exposure to acid-alcohol, and its fluorescence. Further studies of its properties and association with dietary cirrhosis have been made by György and Goldblatt,<sup>2</sup> Blumberg and McCollum,<sup>3</sup> Blumberg and Grady,<sup>4</sup> Edwards and White,<sup>5</sup> György,<sup>6</sup> Popper, György, and Goldblatt,<sup>7</sup> and Endicott and Lillie.<sup>8</sup>

Female rats on a vitamin E-deficient diet develop brownish discoloration of the uterus. The pigment is deposited in the smooth muscle cells, and within large phagocytes in the intermuscular connective tissue (Fig. 1). This very striking and characteristic pigmentation has been studied by Martin and Moore,<sup>9,10</sup> Barrie,<sup>11</sup> Hessler,<sup>12</sup> Demole,<sup>13</sup> Barrie-Sweeten,<sup>14</sup> and by Mason and Emmel.<sup>15</sup> In a recent paper, Mason and Emmel<sup>16</sup> found similar pigment in ovaries and in the interstitial tissue of the testis. They called attention to the resemblance of the pigment to "ceroid" and stated that further studies on its identification were in progress. Moore and Wang<sup>17</sup> and Mason and Emmel<sup>18</sup> have shown that, like "ceroid," the uterine pigment is fluorescent. It has been found in our laboratory and in that of Dr. Mason that the uterine, ovarian, and testicular pigment in vitamin E-deficient rats is acid-fast, and stains selectively with methyl green, thus indicating its "ceroid" character. Similar pigment is present in the motor ganglion cells of the cord and medulla of older rats (Figs. 2 and 3), maintained for long periods on a vitamin E-deficient diet, and is regularly absent in controls receiving wheat germ oil or tocopherol (Table I). We have also noted pigment with identical staining reactions in the atrophic testis of a vitamin E-deficient guinea-pig<sup>18</sup> (Fig. 4). It was located within degenerating germ cells, giant cells, and Sertoli cells of the tubules, but not in the Leydig cells or intertubular stroma.

\* Received for publication, April 19, 1945.

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‡ Personal communication.



In 1917, Whipple and Hooper<sup>19,20</sup> called attention to pigmentation of the intestinal muscularis in dogs with biliary fistulae, and later they noted a similar pigmentation in dogs which had been rendered anemic by bleeding. Nachtnebel,<sup>21</sup> in 1933, re-investigated this pigment, noted a similarity to hemofuscin, and, since the dogs had been fed liver and cod-liver oil, was inclined to attribute it to the absorption of some dietary constituent from this source.

From the microchemical reactions carried out by Nachtnebel,<sup>21</sup> it is impossible to identify the pigment as "ceroid," although the staining with basic fuchsin and Nile blue sulphate is consistent with "ceroid." Through the courtesy of Dr. Karl Mason, we have had opportunity to study a section of dog's intestine from Dr. George H. Whipple's material. The muscle cells were loaded with fuchsinophile, acid-fast gran-

TABLE I  
*"Ceroid" Pigment in Ganglion Cells of Vitamin E-Deficient Rats*

No. of rats	Diet	Age (months)	"Ceroid" pigment			Testicular atrophy
			Brain	Medulla	Spinal cord	
8	—E	7-12½	—	+(8)	+(8)	+
8*	+E	18-20	—	—	—	—

\* Of the controls, 2 received a stock diet; 1, 3 gtt. of wheat germ oil; 1, 6 gtt. of wheat germ oil daily; 4, 5 mg. of synthetic *dl*-alpha-tocopherol weekly.

ules, and these were present also within large phagocytic cells. The appearance was identical with that found in the human cases reported below. Because of its staining with the Ziehl-Neelsen method, this pigment may also be tentatively identified as "ceroid."

It is interesting in this connection that dogs with chronic biliary fistulae have been found by Brinkhous and Warner<sup>22</sup> to develop muscular dystrophy and testicular atrophy, and this is ascribed to failure of absorption of vitamin E when bile is excluded from the intestine. These dogs also, Dr. Mason informs us, contain an abundance of "ceroid" in their intestinal musculature.

So far as we are aware, the occurrence of "ceroid" pigment in human tissues has not been recorded. György<sup>6</sup> made the flat statement that "ceroid" in cirrhotic livers distinguishes the experimental cirrhosis produced in rats by purely dietary means from all other forms of cirrhosis in animals and man. Lillie and his collaborators<sup>23</sup> were unable to find "ceroid" in experimental selenium cirrhosis or in human cirrhosis. Similar negative results were obtained in investigations carried out by

Popper, György, and Goldblatt.<sup>7</sup> More than 200 normal and pathological human livers were examined for "ceroid," including many livers with cirrhosis, acute diffuse necrosis, and hemochromatosis. The only exception to this statement is that in lipoid pneumonia caused by cod-liver oil, the lipoid droplets in the alveolar exudate are known to retain fuchsin after acid decolorization (Pinkerton,<sup>24</sup> Graef<sup>25</sup>). It has been shown recently by Endicott<sup>26</sup> that prolonged oxidation *in vitro* of cod-liver oil or linseed oil with potassium bichromate renders them acid-fast; and Haas<sup>27</sup> and Endicott<sup>26</sup> have found that cod-liver oil injected subcutaneously into rats gradually acquires acid-fastness.

Since the presence of acid-fast "ceroid" pigment in human tissues, other than in the cod-liver oil pneumonias, has not been recognized, it may be of interest to present in detail four cases of profound nutritional disorder in which the tissues, and particularly the intestinal musculature, contained a great abundance of this pigment. We shall refer also to the occurrence and distribution of acid-fast pigment in various tissues from routine autopsy cases. Although no studies of the fluorescence of this pigment have been carried out by us, the assumption that the acid-fast pigment seen in paraffin-embedded material is identical with "ceroid" seems justifiable, and we have used the terms interchangeably. All sections were stained with carbol-fuchsin overnight at room temperature, or at 56° C. for 1½ hours, decolorized with 3 per cent HCl in 80 per cent alcohol for 1 to 3 hours, and counterstained with hematoxylin or methylene blue. In some cases, the pigment was shown to be sudanophilic, and to stain intensely with methyl green, a reaction considered by Popper, György, and Goldblatt<sup>7</sup> to be specific for "ceroid." While the significance of this pigmentation cannot be precisely defined in the light of present knowledge, there is, as will be pointed out later, a certain amount of circumstantial evidence indicating a relationship to deficiency of vitamin E.

### Case 1

M. M., a female, single American school teacher, was admitted to the Presbyterian Hospital (history no. 511705, autopsy no. 14033) four times between February, 1937, and January, 1943.

**Chief Complaint.** The patient's chief complaint was swelling of the legs for the past 5 years. One sister had a similar condition. Three siblings were healthy.

**Present Illness.** About 5 years before admission she began to have swelling of the legs and, about 1 year later, attacks of vomiting before breakfast. There had been occasional episodes of fever and malaise. Later she had edema of the face and scalp, and moderate dyspnea on exertion.

**Physical Examination.** On examination the temperature was 98.2° F.; pulse, 80; respiration, 20. There was edema of the trunk and extremities. Teeth were widely separated and incisors pegged. Skin was inelastic and doughy, with many striae albicantes. Trousseau and Chvostek signs were positive.

*Laboratory Findings.* The venous pressure was 67 mm. of water. Chemical examination of the blood gave the following results:  $\text{CO}_2$ -combining power of the serum, 55.6 vols. per cent; chlorides, 114 mg. per L.; inorganic phosphates, 3.5 mg. per cent; serum protein, 3 per cent; albumin, 1.7 per cent; globulin, 1.3 per cent; calcium, 3.8 mg. per cent; cholesterol, 111 mg. per cent. Hemoglobin was 71 per cent; red blood cells, 3,700,000 per cmm.; leukocytes, 3,160 per cmm. Gastric expression contained no free HCl, 6 per cent combined acids. There was no change after histamine. Phenolsulfonphthalein excretion was 43 per cent after 2 hours. No albumin was found in the urine on numerous examinations. The stools were bulky and fatty at times, but steatorrhea was not a striking feature.

*Course.* During the patient's stay in the hospital, extensive laboratory studies were carried out. Hypoproteinemia and hypocalcemia persisted, but the edema was somewhat relieved, as were the symptoms of active tetany. After her discharge, she was followed in the out-patient department and then referred to the hospital of the Rockefeller Institute for further studies. The underlying mechanism of her disease was not discovered, and she was referred back to the Presbyterian Hospital. Hypoproteinemia and hypocalcemia persisted, the edema increased, and ascites and pleural effusions developed.

### Post-Mortem Examination

The autopsy was performed by Dr. C. Schubert. Only the significant findings need be cited. There was marked subcutaneous edema and little fat. The peritoneal cavity contained 4 liters of pale gray, milky fluid. There were 2800 cc. of similar fluid in the right pleural cavity, 250 cc. in the left. The *lungs* were compressed by fluid, but otherwise normal. The *liver* weighed 1525 gm. and was slightly nodular, particularly in the region of the hilus. The surface was pale, reddish brown, wet and translucent. Connective tissue was slightly increased in the nodular areas. The *spleen* weighed 325 gm., was brick-red and rather soft, and the follicles were indistinct. The *gallbladder* contained a single stone. The aggregate weight of the *parathyroid glands* was 229 mg. The rugae of the *stomach* were well developed and the mucosa was not atrophic. The submucosa of the *small intestine* was edematous, and the muscular coat had throughout a rusty brown color (Fig. 5). The *colon* also was edematous. There were no ulcers and the mucosa grossly was not abnormal. The *heart*, *esophagus*, *pancreas*, *thyroid*, *kidneys*, *uterus*, *tubes*, and *ovaries* showed no abnormality.

### Microscopic Examination

Aside from the edema and the abnormal pigmentation to be described below, no lesions of note were found in any of the tissues.

Pigment was abundantly present in the smooth muscle cells of the lower esophagus, stomach, and small intestine. Both circular and longitudinal coats contained it, but not the muscularis mucosae. Large mononuclear cells stuffed with pigment were seen in the perivascular connective tissue between the muscle bundles and in the subserosa.

The pigment granules, which were coarse, stained yellow brown with sudan III, dark purplish blue with methyl green after differentiation with dilute acetic acid (Popper *et al.*<sup>7</sup>) and brilliant red with carbol fuchsin after decolorization with 3 per cent HCl in 80 per cent alcohol (Fig. 6). The individual muscle cells were distended with the granules, appearing much thicker than normal fibers. The muscle of the large intestine did not contain pigment.

In the liver, the "ceroid" pigment was present in the form of coarse droplets within the Kupffer cells. The liver cells contained fine granules of lipochrome pigment which did not give the characteristic reaction with methyl green or Ziehl-Neelsen stains. The uterine muscle contained it sparingly (Fig. 7). In the ovary, there were large accumulations of phagocytes filled with "ceroid," chiefly in the vicinity of stretic follicles (Fig. 8). Pigment granules were found also in individual muscle fibers in the media of small arteries in the thyroid and pancreas. No pigment was seen in the aorta. The muscle cells of the myocardium, however, showed a considerable amount, having the same distribution as the usual lipochrome pigment, but distinguishable because of its acid-fastness. The individual droplets were larger than the slightly more refractile, brownish lipochrome granules which were also present. The skeletal muscle contained sparse numbers of acid-fast droplets at the poles of the nuclei.

No pigment was found in the muscle of the gallbladder, bronchi, or pancreatic ducts. The spleen, lungs, and adrenals were also free.

### Comment

The nature of this patient's illness, in spite of intensive clinical studies, was never satisfactorily explained. The hypoproteinemia could not be permanently influenced by diets high in protein, administration of casein hydrolysates, amino acids, or high doses of vitamins. It was never clear whether there was a primary failure of absorption, or inability to utilize amino acids for synthesis. There was no history obtainable of vitamin deficiency.

The literature contains isolated reports of cases of so-called "idiopathic hypoproteinemia."<sup>28-33</sup> However, we have found reference to but one autopsy report on such patients, that of Thompson, McQuarrie, and Bell.<sup>30</sup> This patient, a child, 2 years of age, was found to have atrophic changes in the liver, but there is no reference to the condition of the intestines, nor to the presence of pigment.

The following case also illustrates the extensive deposit of "ceroid" pigment which may occur in association with chronic nutritional disorders.

### Case 2

S. B., a taxi driver, 32 years old, was admitted to the hospital (Presbyterian Hospital, history no. 338885, autopsy no. 12281) on four occasions between 1918 and 1936. He first entered because of a duodenal ulcer, diagnosed when he was but 10 years old. A gastro-enterostomy was performed with relief of all symptoms. In 1932, however, he began to have diarrhea and it was shown by x-ray examination that a gastrocolic fistula had developed. From that time until shortly before death, 4 years later, the diarrhea continued, with two to four watery stools daily. He gradually lost strength and weight; there was pitting edema of the ankles and fluid in the abdomen; the cecum and colon became distended. His total serum proteins fell to 4.4 per cent, with the albumin remaining relatively high at 3.3 per cent. There was hypoglycemia. Urine was normal save for an occasional trace of bile. There was no marked anemia.

### Post-Mortem Examination

Autopsy was performed on November 16, 1936, 5½ hours after death, by Dr. Holman. The interesting findings were: (1) a persistent small chronic ulcer near the site of the gastro-enterostomy opening; (2) a double opening of the stomach into jejunum and transverse colon, so that much of the ingested food must have passed directly into the large gut; (3) fatty cirrhosis of the liver which gave the impression of having resulted from continuing destruction and replacement of liver cells—many liver cells showed a peculiar coagulative necrosis of their cytoplasm; (4) advanced emaciation, with almost complete disappearance of the subcutaneous fat.

### Microscopic Examination

*Pigment.* Brownish pigment in the form of granules or droplets retaining the Ziehl-Neelsen carbol fuchsin stain after prolonged treatment with 3 per cent HCl alcohol, and staining dark purplish blue with methyl green, was found in the following situations:

(1) The *smooth muscle* of esophagus, stomach, and small intestine. A section taken through the jejunal-colic fistula showed abundant pigment in the jejunal musculature, but none in the muscle of the large gut except in the immediate vicinity of the line of junction. It was not present in the muscularis mucosae. In the stroma of the mucosa, many plasma cells and large mononuclear cells contained clumps of acid-fast material. Pigment was present also in phagocytes in small lymph nodes and intestinal follicles.

(2) *Skeletal Muscle.* A section of the rectus abdominis muscle, which showed necrosis of some fibers, marked atrophy of others, fibrous replacement, and regenerating myoblasts, showed also abundant acid-fast pigment. This was situated within large macrophages or in spindle-shaped, sometimes multinucleate, myoblasts (Fig. 9).

(3) *Testes.* Spermatogenesis was completely lacking, and many of

the tubules contained only irregular syncytial masses of Sertoli cells. In the fibrillar cytoplasm of these cells were scattered globules or clumps of acid-fast pigment. This, however, was much more abundantly present in the cells between the atrophic tubules. Whether the pigment-containing cells were Leydig cells or large macrophages or connective tissue cells was difficult to determine. Although some of the pigment-bearing cells were in close apposition to the basement membrane, passage of the pigment from tubule to interstitium across the membrane was not observed.

(4) *Liver*. There was advanced fatty infiltration of the liver; the majority of the hepatic cells containing a single large globule. With this there was moderate cirrhosis, rather diffuse and irregular, unaccompanied by marked regeneration of liver tissue or proliferation of bile ducts. Large clumps of acid-fast pigment were present both within the liver cells and in Kupffer cells, and in phagocytes in the cirrhotic stroma. The individual globules varied in size, but were considerably larger than the commonly seen fine granules of lipochrome pigment. They stained brilliantly with the Ziehl-Neelsen stain and with methyl green.

(5) *Kidney*. Clumps of coarse acid-fast pigment were located within the epithelial cells of the loops of Henle.

### Comment

Since much of the food in this patient passed directly from stomach to large intestine, it is fair to assume that intestinal absorption of proteins, fats, carbohydrates, and fat-soluble vitamins must have been greatly impaired. The type of fatty cirrhosis of the liver with abundant accumulation of "ceroid" pigment closely reproduces the experimental cirrhosis of rats induced by low casein diets. Whether the testicular atrophy and dystrophy of skeletal muscle may be interpreted as evidences of vitamin E-deficiency is an open question, since there is no precise knowledge of the effects of vitamin E-lack in human beings. Such a possibility, however, is not to be excluded, and indeed is strengthened by the finding of "ceroid" pigment in tissues where it is deposited in vitamin E-deficient male rats, namely, the testes, smooth and skeletal muscle.

A third case of nutritional disorder, also characterized by pigmentary deposits of this sort, follows.

### Case 3

E. C., an American housewife, 42 years old, was admitted to the Presbyterian Hospital (history no 566263, autopsy no. 13884) on five occasions from November, 1938, to May, 1942. Her history, in brief, was as follows:

*Chief Complaint.* The patient had had diarrhea and weakness since childhood. Since her first pregnancy, 8 years previously, she had suffered from postprandial epigastric pain, soreness of the tongue, and dysphagia. For 3 weeks before admission she had had cramps in the extremities and carpal spasm.

*Family History.* Irrelevant.

*Past History.* The patient had always lived in the United States. Her only previous illnesses had been whooping cough in childhood, psoriasis, and sinusitis.

*Present Illness.* During infancy and childhood, the patient was said to have had dysentery, which was worse during the summer, and to have been anemic. At the age of 28, she felt very weak and was given injections of iron and liver with benefit. During this period, however, she continued to suffer from diarrhea. At the age of 33, she was delivered spontaneously of an 8 months' baby which died within 24 hours. Following this delivery, weakness and diarrhea recurred and, in addition, she complained of a burning sensation in her throat, relieved by vomiting or by lime water. Because of these symptoms and because she wished to have another child, she had consulted Dr. Watson 5 years previously. He found her to have a macrocytic hyperchromic anemia, and also a retroverted, retroflexed uterus. She was admitted and a uterine suspension performed. Upon discharge, she was given liver extract and felt well for 9 months. At that time, she stopped the injections and again developed diarrhea and weakness.

On her next admission in October, 1940, temperature, pulse, and respiration were normal. Her stools were light, semi-liquid and frothy. No ova or parasites were found. She was again given injections of liver extract, with vitamins and one transfusion. Reticulocytes rose to 8.7 per cent and hemoglobin upon discharge was 100 per cent. She remained at home upon a high protein, low fat diet, and was readmitted in April, 1942, for the delivery of a full-term child, which survived. Two weeks later, diarrhea again became severe, she became weak and suffered from numbness, cramps, and twitchings of the extremities. She was again admitted to the hospital.

*Physical Examination.* Temperature, pulse, respiration, and blood pressure were normal. During examination, she displayed typical symptoms of tetany. She became dehydrated in spite of vigorous intravenous therapy. The blood calcium, which was 6.3 mg. per cent on admission, was raised to 8.7 by administration of calcium gluconate.

The blood protein was never greatly reduced. On her last admission, total proteins ranged between 5.85 and 7.26 per cent, globulins between 1.45 and 2.00, albumin between 4.40 and 5.26. There was thus no significant disturbance of the albumin/globulin ratio. The other laboratory tests threw no further light upon the case. She failed rapidly and died. The diagnosis was *non-tropical sprue*.

### Post-Mortem Examination

At autopsy, which was performed by Dr. Beaubien 2 hours after death, there was no edema nor fluid in the serous cavities. All thoracic and abdominal viscera were grossly normal. The intestines were distended with gas; the Peyer's patches were atrophic; there were no ulcers. The colon also was dilated, but not otherwise abnormal.

### Microscopic Examination

Pigment, retaining fuchsin after acid decolorization, was found in abundance in the smooth muscle fibers and within large phagocytic cells in the *small intestine*. The *liver* also contained a considerable amount of

pigment, both within hepatic cells and in the Kupffer cells. Some of the granules in the liver cells appeared angular and had a brownish cast; others stained pure red and had the form of droplets. The pigment in the Kupffer cells stained pure red. In the *uterus* there was acid-fast pigment in moderate amount in many of the smooth muscle cells, as well as within large mononuclear phagocytes. These were especially numerous in the stroma of the mucosa. In the *ovary* large collections of "ceroid"-containing phagocytes were seen in the vicinity of vessels and about atretic follicles. The pigment in the reticular zone of the *adrenal* cortex retained the fuchsin after decolorization. There were scattered *skeletal muscle* fibers showing hydropic vacuolar degeneration without reaction. No pigment was found in the muscle.

### Comment

This woman suffered from diarrhea with sprue-like stools over many years, associated with macrocytic hyperchromic anemia and hypocalcemia. During her final illness, however, there was no anemia, plasma proteins were within normal limits, and there was no edema.

Bearing on the possibility of a vitamin E deficiency is the death of her first child 24 hours after an 8 months' delivery. The peculiar hydropic degeneration of the skeletal muscle appears to be a terminal change and cannot be regarded as significant.

### Case 4

A. U., female, Irish housemaid, single, age 33, was admitted (Research Division, Goldwater Memorial Hospital: history no. 772, autopsy no. A1026) on July 1, 1943, and died on February 6, 1944.

*Chief Complaints.* The patient complained of swelling of the legs since December, 1941; weight loss, weakness, abdominal cramps, and diarrhea.

*Family History.* Irrelevant.

*Past History.* There had been a tonsillectomy for recurrent tonsillitis in October, 1941.

*Present Illness.* With the onset of edema of the ankles and general weakness in 1941, the patient went to St. Vincent's Hospital, remaining there for 8½ months. Menses ceased 1 month after admission. Painless diarrhea with bulky, watery stools began 2 weeks after her entrance to the hospital and continued until her death. In February, 1943, she was admitted to Bellevue Hospital and in April to the Presbyterian Hospital. At this time, there was no dependent edema. In May, she was sent to the Goldwater Memorial Hospital. She had lost 42 pounds from the onset of her illness to the time of last admission.

*Physical Examination.* Emaciation, pallor and tenderness in right lower quadrant near iliac crest.

*Laboratory Findings.* Examination of the blood gave the following data: Red blood cells, 2.7 to 3.0 millions; white blood cells, 7,550 with 10 per cent eosinophils; reticulocytes, 3.5 per cent; serum cholesterol, 191 mg. per cent; serum albumin, 2.1 gm. per cent; serum globulin, 2.3 gm. per cent; bromsulfalein retention, 0 at ½ hour; serum chlorides, 339 mg. per cent; CO<sub>2</sub>-combining power, 60 vols. per



cent; blood calcium, 7.8, 7.1, and later, 10.4 mg. per cent; blood phosphates, 3.9 mg. per cent. The urine had a specific gravity of 1.016 to 1.026; albumin was 1 plus; there were no red or white blood cells or casts. Phenolsulfonphthalein excretion was 87 per cent in 2 hours. Guaiac test of the stool was negative; the fat content was 8.6 to 21.0 per cent.

### Post-Mortem Examination

Autopsy was done by Dr. J. Victor, 2½ hours after death. The essential gross findings were chronic jejunitis, chronic mesenteric lymphadenitis, emaciation, anemia, osteoporosis, fatty liver, Laënnec's cirrhosis, thrombosis of portal vein (intrahepatic), splenomegaly, anasarca, purpura, hemorrhage in renal pelvis, squamous metaplasia in pancreatic ducts, lobular pneumonia, involution of ovary.

### Microscopic Examination

Many signs of malnutrition are included in the diagnoses above. Intracellular granules or droplets retaining carbol-fuchsin stain after prolonged acid decolorization were present at various sites, namely, within histiocytes of mesenteric lymph nodes, germinal follicles of spleen, fibrous scars within the liver, stroma of uterine endometrium, and in the infiltrated and scarred jejunal lesions. Acid-fast granular material was found also in smooth muscle cells of arteries of the spleen and ovary, and in the intestinal muscle of the jejunum near the lesions. It was found also within endothelial cells of capillaries, veins, and arteries in the ovary, and in the myoepithelial cells of the juxtaglomerular bodies of the kidneys.

### Comment

This woman suffered from diarrhea, probably due to chronic jejunitis, over a period of 3 years. This resulted in wasting, hypoproteinemia, and undoubted impairment of intestinal absorption. The hepatic changes closely resemble those produced experimentally with low protein diets. There was widespread deposit of "ceroid" pigment in the locations cited.

### DISTRIBUTION OF ACID-FAST PIGMENT

In view of the presence of large amounts of acid-fast pigment in these cases of obvious nutritional deficiency, it seemed imperative to search systematically in different tissues for its occurrence. Pigment having this property of "acid-fastness" has been found in many cases and in various situations, as will become apparent from the following survey.

*Heart.* The juxtannuclear lipochrome pigment has been found to vary

in its acid-fast staining. In 42 human hearts examined, the pigment was distinctly brown and non-acid-fast in 12 cases, brown with a reddish cast in 16, and distinctly red in 6. It is interesting that the cases in which the pigment had a "ceroid" character were all associated with liver diseases, namely, hemochromatosis with cirrhosis (2) or Laënnec's cirrhosis (4), one of the latter group having, in addition, carcinoma of the stomach. When the pigment was acid-fast, it appeared to occur in larger masses or even as thick rods with rounded ends.

*Aorta.* Phagocytes filled with brilliantly red-stained, acid-fast droplets have been noted among the foam cells of atherosclerotic plaques and in medial syphilitic scars.

*Blood Vessels.* Acid-fast pigment droplets have been found in the muscle cells of medium-sized arteries of thyroid, pancreas, spleen, and ovaries. They have also been found within the endothelial cells of venules, capillaries, and lymphatics.

*Lymph Glands.* Acid-fast pigment may occur within large phagocytic cells.

*Spleen.* In two cases of hepatic cirrhosis and one of lymphatic leukemia with extensive infiltration of the small intestine, phagocytes containing "ceroid" were found within the malpighian follicles.

*Alimentary Tract.* Except for the cases reported, no pigment was found in the esophagus, and only twice in small amounts in the muscle cells of the stomach in a case of Laënnec's cirrhosis, and in a child with cystic fibrosis of the pancreas. In addition to the above cases, pigment has been found in the muscle fibers of the small intestine in 4 of 27 cases. The positive cases were of celiac disease (1), Laënnec's cirrhosis (2), and cystic fibrosis of the pancreas (1). In 18 sections of large intestine, no pigment was found. In 1 case, it was present in the lymphoid tissue of the appendix, and in another, in leukemic infiltrations of the ileum.

*Liver.* The question of the occurrence of "ceroid" in this organ is of particular interest in view of the negative reports of others. In our material, acid-fast pigment could be demonstrated within liver cells, in Kupffer cells, within phagocytes in the scarred areas of cirrhotic livers, and within endothelial cells of small blood or lymph channels (Fig. 10). We have examined 38 cases of Laënnec's cirrhosis, and have found acid-fast pigment in 21, an incidence of 55 per cent. Included in this group are 4 cases of primary liver cell carcinoma. No acid-fast pigment was detected in the neoplastic tissue, although in 3 cases it was abundantly present in the noncancerous tissue. In 6 cases of coarse nodular cirrhosis, 3 showed the pigment. One of the positive cases was clearly due to cinchophen. A case of nontropical sprue had a fatty liver in

which many large phagocytes were filled with coarse acid-fast droplets. Liver sections from a case of hemochromatosis, with a history of recovery from sprue, also contained both "ceroid" and hemosiderin. Two other cases of hemochromatosis did not show pigment in the liver. In 2 of 7 cases of cardiac cirrhosis, pigment was present. Five cases of chronic passive congestion contained none, nor did 11 normal livers.

As in the heart muscle, the lipochrome pigment of liver cells and Kupffer cells often exhibits some degree of acid-fastness. In the cases referred to as positive, only pigment showing brilliant red staining has been included. In 12 miscellaneous cases in which the liver was not diseased, no acid-fast pigment was found. Sections from 6 gallbladders also showed no pigment.

*Pancreas.* No acid-fast pigment was found in the pancreas in 20 cases examined.

*Adrenals.* The pigment normally present in the reticular zone of the adrenal cortex is in some cases definitely acid-fast. This was true in 7 of 22 sections examined. The diagnosis in these cases was nontropical sprue (1), leukemia with hemochromatosis (1), and Laënnec's cirrhosis (5). In 5 of the remaining cases, the pigment was brownish red, and in the other 10 there was no retention of carbol-fuchsin after acid decolorization. When present, "ceroid" occurred not only in the adrenal cortical cells, but also within phagocytes in the sinuses.

*Kidney.* In the kidney the pigment has been seen in two situations: in the juxtaglomerular bodies, in the form of minute granules in the myoepithelial cells; and in the descending loops of Henle. Here the brownish pigment which is so commonly present may in some cases be strongly fuchsinophilic. This appears to be exceptional, and not correlated with the deposit of acid-fast pigment in other tissues.

*Urinary Bladder.* No pigment has been seen in the muscle of the urinary bladder, even in those cases in which it was abundantly present in the intestinal muscle.

*Prostate.* No pigment was noted in the prostate of 8 cases.

*Testis.* The testes from 38 cases were studied. "Ceroid" was conspicuous and abundant in the interstitial cells in 19; in the remaining 19 the pigment showed little or no acid-fastness. It was usually, though not invariably, associated with atrophy of the spermatogenic epithelium and was contained both in the Leydig cells and in phagocytes near the tunica. An interesting finding was the presence of large droplets of "ceroid" in the interstitium of the testis of a 10-weeks-old infant dying of toxoplasmosis. Droplets of "ceroid" were occasionally seen among the Sertoli cells of atrophic tubules and in the epithelial cells of the rete testis and epididymis.

*Seminal Vesicles.* The epithelium and subjacent connective tissue cells of the seminal vesicles normally contain an abundance of yellowish brown pigment, the nature of which has not been defined. In 3 of 11 sections it proved to be strongly acid-fast. The pigment was present in both the epithelial cells and in isolated cells in the underlying connective tissue (Fig. 12).

*Ovary.* Our material includes sections of ovary from 12 cases. Phagocytes laden with acid-fast pigment were found in 6, usually in the vicinity of atretic follicles. In one case there was massive accumulation of the pigment, together with hemosiderin, in the wall of a small cyst.

*Uterus.* In addition to the case cited above in which the pigment was present in smooth muscle cells of the uterus, pigment was found in endometrial phagocytes in one case of Laënnec's cirrhosis.

*Skeletal Muscle.* In one case of fatty cirrhosis in an alcoholic patient, "ceroid" was present at the poles of the myolemmal nuclei. The muscle fibers were otherwise normal.

*Thyroid Gland.* In 18 cases no acid-fast pigment was found in the thyroid gland.

*Lung.* No acid-fast pigment was found in the lungs.

*Pituitary Gland.* Much of the brownish pigment normally present in the pars nervosa of the pituitary gland is acid-fast. In a case of hemochromatosis, it was deposited in very large amounts along with granules of hemosiderin (Fig. 11).

*Central Nervous System.* The occurrence and distribution of acid-fast pigment in the central nervous system has been the subject of a separate communication.<sup>40</sup> It may be stated here that such pigment may be found in abundance in the ganglion cells of cortex, thalamus, medulla, and spinal cord, in microglia and oligodendroglia, and in endothelium and perivascular phagocytes.

## DISCUSSION

In trying to interpret the possible significance of this pigment, one is handicapped by the fact that the term "ceroid" has no precise chemical implication. Three substances have been obtained by Reeves and Anderson<sup>34,35</sup> from avian and human tubercle bacilli which are strongly acid-fast. They have been identified as optically active hydroxyacids of high molecular weight. Unlike "ceroid," they are soluble in  $\text{CHCl}_3$  and ether, so that if similar substances are present in the tissue "ceroids," they are probably in combination with substances which are insoluble in lipid solvents. It may well be that the property of acid-fastness is not restricted to a single chemically defined substance, and that in the case of the commonly occurring lipochrome or "wear and

tear pigments" one is dealing with a mixture of substances. One may indeed find granules of brown and red pigment intimately commingled in the same cell, and one may observe all transitions in staining between brown and pure red. As a rule, the red droplets are somewhat larger and more irregular in shape than the brown granules. This is particularly true in heart muscle.

What then may be said as to the significance of this pigment? Our own observations and a study of the literature indicate that "ceroid" is found in association with the following conditions:

1. Vitamin E deficiency of rats and other laboratory animals.
2. Dietary cirrhosis in rats, and cirrhosis produced by other means in rats and other laboratory animals.
3. Chronic biliary fistulae and experimental anemia in dogs.
4. Nutritional disorders in man, in which there has been presumptive impairment of absorption by the small intestine.
5. Cirrhosis of the liver and hemochromatosis in man.
6. Focal degenerative lesions.

The "ceroid" deposition which results from dietary lack of vitamin E can be prevented by administration of that vitamin. Although there is no precise knowledge of its derivation or the chemical reactions involved in its deposition, the relation to uncomplicated vitamin E deficiency is established.

In the dietary cirrhosis of rats produced by low protein diets, the presence of large amounts of "ceroid" pigment in the liver has been regarded as a distinctive feature of the hepatic lesion. It has been attributed to break-down products of the damaged liver cells or, more recently, to some derivatives of cod-liver oil in the experimental diets. That an associated vitamin E deficiency may be concerned, has not been considered; the possibility, however, merits discussion.

It is interesting that, with few exceptions, the experimental diets used in the production of hepatic cirrhosis have been obviously deficient in vitamin E. Thus, Lillie, Daft, and Sebrell<sup>1</sup> used a diet in which the only source of tocopherol was 3 per cent of Wesson oil.\* Of the diets used by György, only one (diet C 1) contained adequate vitamin E, in the form of 38 per cent hydrogenated cotton-seed oil. Blumberg and Grady<sup>4</sup> found less "ceroid" when the basal diet was supplemented with 3 to 5 cc. of wheat germ oil than was shown by the rats of Blumberg and McCollum<sup>3</sup> which received no vitamin E.

\* With an average food consumption of 10 gm. and a value of 64 mg. of tocopherol per 100 gm. of Wesson oil (as determined by Dr. Hans Kaunitz in our laboratory), one may calculate a daily intake of 0.194 mg. of tocopherol. This would be below the normal daily requirement.<sup>20</sup>

Bearing further on this question is a recent paper by Endicott, Daft, and Sebrell,<sup>37</sup> in which they report the production of dietary cirrhosis without "ceroid." Although the authors do not stress the point, it is interesting to note that their rats received a weekly supplement of 3 mg. of alpha-tocopherol. We have examined cirrhotic livers of 21 rats for "ceroid." Acid-fast granules and globules were found in 20, the cirrhosis having been produced by low protein diets, prolonged poisoning with carbon tetrachloride or excess dietary l-cystine, or cysteic acid. In the 5 per cent l-cystine group, acid-fast pigment was strikingly more abundant in 15 rats given a vitamin E-deficient diet than in 15 rats receiving McCollum's stock diet.<sup>38,39</sup>

Further experiments are needed to clear up this question.

"Ceroid" pigmentation of the muscle of the small intestine takes place in dogs with long-standing biliary fistulae, which, as Brinkhous and Warner<sup>22</sup> have shown, also develop muscular dystrophy and atrophy of the testes. It is reasonable to assume that failure to absorb vitamin E from the intestines is the determining factor in producing these characteristic lesions.

In regard to the anemic dogs from Dr. Whipple's laboratory, positive evidence is lacking that vitamin E deficiency was responsible for the "ceroid" deposit in the intestinal musculature. However, the administration of large amounts of cod-liver oil is known to favor destruction of vitamin E; the dogs examined by Nachtnebel<sup>21</sup> received from 10 to 15 cc. of cod-liver oil daily in addition to that incorporated in the salmon bread which formed a large part of their basal diet. Indeed, the only possible source of vitamin E was that which may have been contained in cooked pig's liver.

In the four human cases reported here, in which there was abundant "ceroid" in the intestinal muscles and elsewhere, there is every reason to assume a severe impairment of intestinal absorption. There is, of course, no direct evidence that the ability to absorb vitamin E was in abeyance, but the muscular dystrophy and atrophy of the testes in case 2 present an interesting analogy to the lesions in dogs with biliary fistulae, which, as has been stated, may with some probability be ascribed to deficiency of vitamin E.

In cases of hepatic cirrhosis and hemochromatosis, in which acid-fast pigment was frequently found, one cannot without further knowledge implicate vitamin E deficiency as a factor. If it is proved that lack of vitamin E is responsible for the accumulation of "ceroid" in the hepatic cirrhosis of rats, it will seem logical to ascribe its occurrence in human cirrhosis to a similar deficiency.

Of the occasional finding of "ceroid" about focal degenerative lesions, such as atheromatous plaques, atretic ovarian follicles, or encephalomalacic areas in the brain, one can say little other than to record its occurrence in such situations.

### SUMMARY

Acid-fast pigment, "ceroid," has been demonstrated in various tissues including the livers of certain human cases of nutritional disorders and hepatic disease. There is accumulating evidence which suggests a possible relation of this pigmentation to deficiency of vitamin E.

We wish to express our thanks to Dr. Walter W. Palmer and Dr. Allen O. Whipple for permission to use clinical records, and to Mrs. Claudia Schogoleff and Miss Goldie Spierer for technical assistance.

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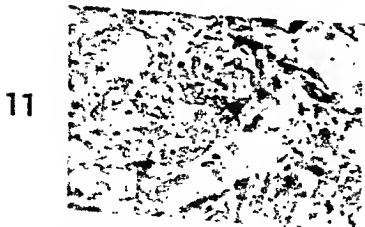
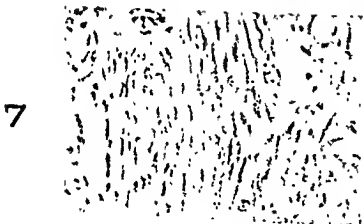
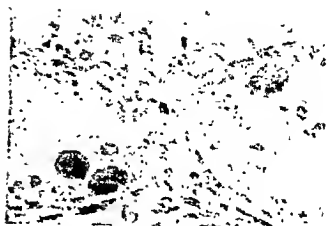
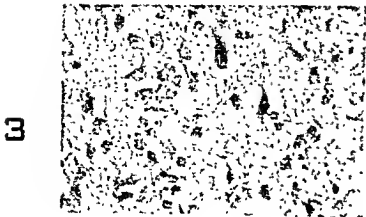
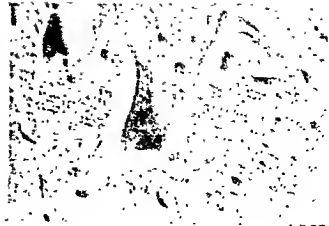
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### DESCRIPTION OF PLATE

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#### PLATE 87

- FIG. 1. Uterus of rat on vitamin E-deficient diet. "Ceroid" in smooth muscle and phagocytes.  $\times 125$ .
- FIG. 2. Rat 431, 8 months on vitamin E-deficient diet. Anterior horn cell of spinal cord containing mass of "ceroid" in cytoplasm.  $\times 450$ .
- FIG. 3. Rat 3134M, 7 months on vitamin E-deficient diet. "Ceroid" pigment in medulla.  $\times 450$ .
- FIG. 4. Guinea-pig. Atrophic testis following 175 days on vitamin E-deficient diet. "Ceroid" within atrophic tubules.  $\times 450$ .
- FIG. 5. Case 1 (autopsy 14033). Small intestine showing brown pigmentation of muscularis.
- FIG. 6. Case 1. Small intestine. "Ceroid" pigment in muscle cells and phagocytes.  $\times 100$ .
- FIG. 7. Case 1. Uterus. "Ceroid" in muscle fibers.  $\times 450$ .
- FIG. 8. Case 1. Ovary. Phagocytes containing "ceroid" in stroma.  $\times 450$ .
- FIG. 9. Case 2 (autopsy 12281). Skeletal muscle, showing atrophy. "Ceroid" in myocytes and phagocytic cells.  $\times 450$ .
- FIG. 10. Cirrhosis of liver (human). "Ceroid" in liver cells and phagocytes.  $\times 450$ .
- FIG. 11. Hemochromatosis (autopsy 14460). "Ceroid" in pars nervosa of pituitary gland.  $\times 450$ .
- FIG. 12. Cirrhosis of liver (autopsy 14342). Seminal vesicle. "Ceroid" in epithelial cells and fibrocytes of stroma.  $\times 450$ .





## STUDIES ON CHANCROID

### I. OBSERVATIONS ON THE HISTOLOGY WITH AN EVALUATION OF BIOPSY AS A DIAGNOSTIC PROCEDURE \*

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This report is the first in a series of studies dealing with the clinical, bacteriologic, and histologic aspects of chancroidal infection. In this paper the histologic appearance of chancroid is described and biopsy as a diagnostic procedure is discussed.

There is definite need for a simple and accurate laboratory method by which the diagnosis of chancroid can be established.<sup>1,2</sup> This has been emphasized recently by the statement from the Surgeon General of the United States Army that the usual laboratory tests are not recommended.<sup>3</sup> The Ducrey skin test was thought to be diagnostic, but Knott and his associates<sup>4</sup> have shown that this test is of limited value. Auto-inoculation is not generally used in the diagnosis, since it produces a new infection. The identification of the Ducrey bacillus is the most accurate method of diagnosis but the demonstration of the organism in direct smears of the lesion has many pitfalls,<sup>5</sup> and isolation by culture is not generally attempted.

In this study cultures were taken from all lesions from which tissue had been obtained for biopsy. The identification of the Ducrey bacillus confirmed the histologic diagnosis in our cases and offered, at the same time, an opportunity to compare the usefulness of culture and biopsy. To our knowledge no similar studies have been reported in which a large group of cases were investigated concurrently by histologic and bacteriologic methods.

#### METHOD OF STUDY

The histologic study is based upon 45 specimens taken for biopsy, which were diagnosed as chancroid. They occurred among 59 specimens taken from 125 patients who had been selected for a special study of lymphogranuloma venereum and chancroid. These patients were subjected to a number of diagnostic procedures which included skin tests, microscopic examination of stained smears, darkfield preparations, lymphogranuloma complement-fixation tests, serum protein determinations, biopsy, auto-inoculation, culture, and inoculation of mice and chick embryos.

\* Aided by a grant from the Venereal Disease Division of the United States Public Health Service.

Received for publication, April 30, 1945.

Tissue specimens were taken without anesthesia with a Gaylors biopsy forceps from the base of the ulcer, usually with little pain. Care was taken to avoid the adjacent skin, and bleeding was easily controlled by local pressure. The specimens averaged 3 mm. in diameter; they were fixed immediately in Zenker's fluid containing 5 per cent glacial acetic acid. Mallory's phloxine-methylene blue stain was used routinely, but in some instances Giemsa's and Gram's stains were also used. The histologic diagnoses were made within 24 hours, and were returned before the results of the cultures were known.

Cultures were taken from the primary lesion or from the auto-inoculation in every case. A modification of the Teague and Deibert method<sup>6</sup> was used and the culture was reported as positive only when the typical tangled chains of gram-negative coccobacilli, the Ducrey bacillus, were found. A discussion of the technic of culture is to be reported elsewhere.

There were 28 male and 17 female Negro patients in this series. The lesions varied in duration from 2 days to 4 weeks. In two instances the lesions occurred in the upper vagina or on the cervix, while in the remainder the lesions were located on the external genitalia.

### HISTOLOGIC OBSERVATIONS

Little information on the histologic appearance of chancroid is found in the standard textbooks of pathology, although a description of some features is given by Moore.<sup>7</sup> He described an intra-epidermal abscess extending into the dermis, perivascular infiltration by polymorphonuclear leukocytes and a few plasma cells, dilatation of blood vessels and lymphatics, and marked acanthosis of the epithelium at the edge of the ulcer.

According to McCarthy,<sup>8</sup> the "most typical histologic picture" is an ulcer which is generally confined to the upper corium, but may extend into the subcutaneous tissues. There is moderate acanthosis of the epithelium. Lymphocytes and plasma cells participate to an approximately equal degree in the diffuse and dense inflammatory infiltration. Polymorphonuclear leukocytes predominate on the ulcerated surface. The inflammatory cellular infiltration blends with the adjoining tissue where it forms perivascular cuffs. The blood vessels and lymphatics are dilated and the former show marked endovasculitis and perivasculitis.

Pund, Greenblatt, and Huie<sup>9</sup> described similar changes and stressed the marked swelling of the endothelium which obstructs the capillaries and leads to necrosis. They considered the vascular changes as "characteristic and responsible for the superficial necrosis and unhealthy granulation." Von Haam<sup>10</sup> felt that these changes represent only a

superimposed fusospirochetal infection. In his observations he noted the absence of any vascular lesions and stated that the histologic picture of chancroid is characterized by the absence of specific changes. Greenblatt<sup>5</sup> considered the histologic changes as suggestive of chancroid, but not sufficiently distinct to permit the diagnosis on the basis of biopsy alone.

Our observations are based upon small specimens, most of which were taken for biopsy from fully developed or persistent lesions. No attempt will be made here to follow the sequences of formation in this lesion. However, the comparatively large number of specimens in this series, as well as 65 additional specimens of various genital lesions obtained from sources outside this study, made possible the development of certain general concepts. Our conclusions were further supported by independent bacteriologic studies of the same lesions.

The following description represents a composite picture as reconstructed from the study of numerous specimens, and refers to an active lesion of about 2 to 3 weeks' duration.

The lesion rarely extends more than 2 or 3 mm. in depth and under low magnification shows two or three zones which blend into each other (Fig. 1). There is generally a rather shallow surface zone of necrotic tissue representing the base of the ulcer. Below this is a fairly wide layer of edematous tissue with numerous blood vessels which are prominent and dilated. Their walls are thin and it is obvious that they are newly formed. In properly oriented sections the blood vessels display a vertical or palisade-like arrangement in their relation to the ulcerated surface. This zone leads into the deep layer in which there is a marked and diffuse cellular infiltration. Here the blood vessels, although still numerous, are less conspicuous and without any particular arrangement. The cellular infiltration fades into the adjacent edematous tissue where it persists around the dilated blood vessels.

On close observation the surface zone consists only of necrotic tissue, red blood cells, some fibrin and large numbers of neutrophilic polymorphonuclear leukocytes. Microorganisms are common and persistent search may reveal gram-negative coccobacilli between the cells of the surface zone, which are morphologically consistent with the Ducrey bacillus.

The mid-zone of the lesion is cellular. It is evident that the majority of cells in this zone are endothelial cells in varying stages of proliferation (Fig. 2). These outnumber all other cells. They are seen as apparently single elements or in small groups, as solid cords or buds originating from a capillary, and as already partially patent channels. Where the mid-zone joins the surface layer, the endothelial cells are

undergoing necrosis (Fig. 3); elsewhere they develop into capillaries. No microorganisms can be seen within these cells.

Marked endothelial proliferation is also seen in both the pre-existing and newly formed vessels in which the endothelium tends to encroach upon the lumen. The process involves arterioles, veins, and capillaries (Fig. 4). Fibrinoid degeneration of the vessel wall, margination and infiltration by neutrophilic polymorphonuclear leukocytes, and sometimes thrombosis are seen near the surface layer (Figs. 3 and 5).

The interstitial connective tissue is edematous. The lack of significant proliferation of fibroblasts in the presence of the marked endothelial overgrowth is striking (Fig. 2). Toward the surface a moderate number of neutrophilic polymorphonuclear leukocytes are present, while plasma cells appear in the deeper portions.

The deep zone of the lesion displays a marked infiltration by plasma cells and less numerous lymphocytes. This infiltration is diffuse and not particularly perivascular. The numerous blood vessels show well formed and thick walls. Endothelial proliferation is less marked and is largely confined to the lumen of the vessels. No degeneration of vessel walls with leukocytic infiltration or thrombosis is encountered.

The tissue surrounding the lesion is edematous and shows some fibroblastic proliferation. The blood vessels are dilated and their endothelium is prominent. There is some perivascular cuffing with lymphocytes and plasma cells. The lymphatics are markedly distended with granular eosinophilic material, some fibrin, and varying numbers of white blood cells. Occasionally, fibrin thrombi are found in the lymphatics which then may show appreciable endothelial proliferation.

The epidermis at the edge of the ulcer is necrotic and more peripherally shows edema, acanthosis, and some polymorphonuclear leukocytic infiltration. There is no repair. The inflammatory cellular infiltration extends for some distance beneath the epidermis on the sides of the ulcer.

In lesions of about 1 week's duration the findings are similar. Here, however, the surface zone of necrosis and ulceration is wide as compared with the mid-zone or the deep layer. The mid-zone displays the same striking endothelial overgrowth without proliferation of fibroblasts which has already been described. Necrosis of the vessel walls with leukocytic infiltration and thrombosis is frequent. The deep zone is poorly developed and there is relatively slight infiltration by plasma cells and lymphocytes.

In lesions older than 3 weeks, or when there is no further evidence of spreading, lymphocytes increase in the deep zone and eventually

outnumber the plasma cells. Sometimes distinct lymphoid follicles are formed. Plasma cells and, later, lymphocytes extend upward into the mid-zone. Healing takes place by proliferation of fibroblasts extending from the deep zone between the numerous blood vessels of the mid-zone (Fig. 6). Collagen is deposited, and some of the blood vessels, pinched off by connective tissue, undergo atrophy. By now the vessels have acquired thick walls. An increased number of endothelial cells line the vessels, but endothelial proliferation has largely subsided (Fig. 7). The base of the ulcer is filled in this manner and the epithelium at the edge of the lesion regenerates.

The same picture is seen not only in the primary lesion, but also in tissue from an area of auto-inoculation or from the wall of a ruptured bubo.

When the specimen includes tissue from all parts of the lesion the recognition of chancroid is not difficult. In many instances, however, the specimen is taken from the base of the ulcer and may show only the surface and mid-zone, with little of the deep layer and nothing from the edge or the surrounding tissues included. Even then the findings are sufficient to permit the diagnosis.

The demonstration of gram-negative coccobacilli in the section is not considered diagnostic, since it is subject to the same uncertainties as the direct smear.

### RESULTS

The histologic diagnosis of chancroid was made in 45 of 59 specimens taken for biopsy. The diagnoses in the remaining 14 cases were: lymphogranuloma venereum (7), granuloma inguinale (2), syphilis (2), and nonspecific inflammatory process (3). Since this investigation was intended to study chancroid and lymphogranuloma venereum, the preponderance of chancroidal infections in our biopsy material was to be expected. The routine material obtained from sources other than this series showed the usual incidence of venereal diseases.

The diagnosis of chancroid was made by biopsy in 45 cases, 35 of which were confirmed by culture. Biopsy yielded the correct diagnosis in all but one of the cases proved by culture. In this instance the Ducrey bacillus was cultured from the lesion, but biopsy showed granuloma inguinale with Donovan bodies.

There were 10 cases in which the culture for the Ducrey bacillus was negative but biopsy revealed chancroid. In all of these 10 cases the clinical picture was consistent with the diagnosis of chancroid.

Three patients in the group of 59 appeared clinically to have chancroid, but the specimens for biopsy showed only a nonspecific picture and the cultures were negative.



## COMMENT

The histologic diagnosis of chancroid is based upon a number of findings, which taken individually are not characteristic but when found together may permit the diagnosis. There is the general architecture of the lesion consisting of an ulcerated surface and one or two deeper layers. The blood vessels display one of the most striking features. There is marked endothelial proliferation which in its diverse aspects occurs predominantly in the mid-zone. Here the endothelium in the various stages of overgrowth outnumbers all other cells. In the same area the lack of appreciable fibroblastic proliferation constitutes another important finding. The palisading of blood vessels, with degeneration of their walls and occasionally thrombosis, is part of the peculiar vascular pattern. There is finally the dense infiltration of the deep zone by plasma cells and lymphocytes with a gradual transition into the surrounding tissues.

The histologic picture can be differentiated from that of other genital lesions. It can be distinguished with reasonable accuracy from non-specific inflammatory processes by following the criteria which have been outlined. Syphilis must be excluded, but its histologic picture is sufficiently well known to separate it from chancroid. Little is known about the histologic picture of the primary lesion in lymphogranuloma venereum, but in our experience it does not seem to bear any resemblance to chancroid. Granuloma inguinale is easily ruled out, for the general pattern is different and that diagnosis can be established by demonstrating the Donovan bodies.

The Ducrey bacillus was not cultured in 10 of the 45 cases in which a diagnosis of chancroid was made by biopsy. Two of these 10 lesions were on the upper vaginal mucosa and cervix, while in another instance the material was from a ruptured bubo. Because of the location and character of these lesions, the cultures were heavily contaminated. It has been our experience that the presence of other bacteria interferes with the growth of the Ducrey bacillus. The clinical appearance of the remaining 7 cases was entirely consistent with chancroid.

In this study we have encountered but one instance of mixed venereal infection in the same lesion. In this case, as already mentioned, the Ducrey organism was cultured from the lesion but biopsy showed the histologic picture of granuloma inguinale with typical Donovan bodies and no evidence of chancroid. Concurrent lymphogranuloma venereum was noted in 2 cases. In 5 other cases syphilis developed while the patients were under observation.

The diagnosis of chancroid by biopsy appears to have certain advantages. In our experience, securing tissue for biopsy is a simple procedure and does not interfere with healing of the lesion. For a variety of reasons the diagnosis is made more often by histologic examination than by culture. Although one tissue specimen is usually sufficient, two or three attempts to culture the lesion were sometimes necessary before the Ducrey bacillus could be identified. In fact, in 2 cases the Ducrey organism was obtained only from auto-inoculation lesions and not from the primary. Examination by biopsy seems particularly useful for those lesions which, because of location or long duration, are heavily contaminated by other bacteria. Present cultural methods usually require more than 24 hours of incubation, while our histologic diagnoses are routinely returned within this period. Securing tissue for biopsy, however, is a painful procedure in extremely small or early lesions and in such cases it is not a practical procedure. Fortunately, diagnosis by culture and smear is usually possible under such circumstances because of fewer contaminating bacteria.

In the course of this study the clinical diagnosis was changed in a number of cases by biopsy. Histologic examination not only confirmed other laboratory procedures but was also valuable when other methods failed. Early carcinoma, granuloma inguinale, and primary syphilis have been diagnosed on histologic evidence alone.

### CONCLUSIONS

The histologic character of chancroidal infection was studied in specimens taken for biopsy from 45 patients. They formed part of a series of 125 cases selected for a special study of chancroid and lymphogranuloma venereum. In 35 of these 45 cases, the histologic diagnosis of chancroidal infection was confirmed by culture of the Ducrey bacillus obtained from the same lesions. The organism was not cultured from the other 10 cases, in which, however, the clinical picture was consistent with the diagnosis of chancroid.

The histologic picture of chancroid is sufficiently distinct to permit diagnosis and to differentiate this condition from other genital lesions. Useful features are the zonal character of the inflammatory reaction, the marked endothelial proliferation in the mid-zone, the meager fibroblastic response at the same level, and the dense infiltration with plasma cells and lymphocytes in the deepest zone. The advantages of biopsy in the diagnosis of chancroidal infection are such that this procedure is suggested as a practical diagnostic tool.

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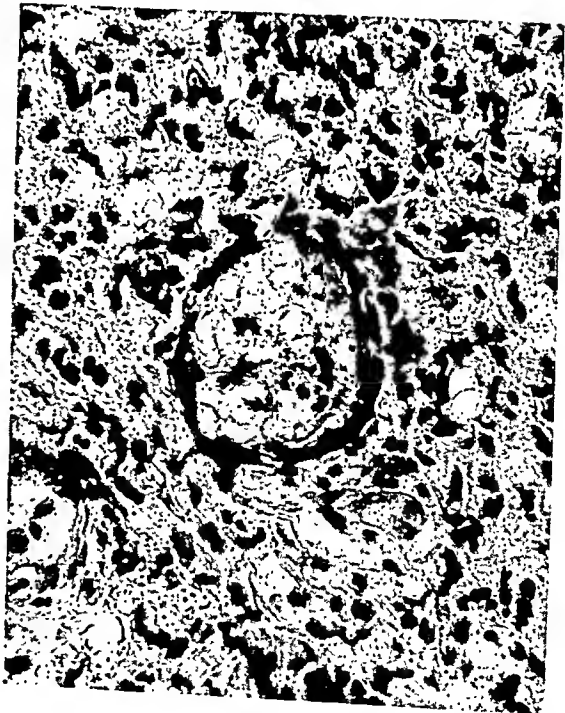
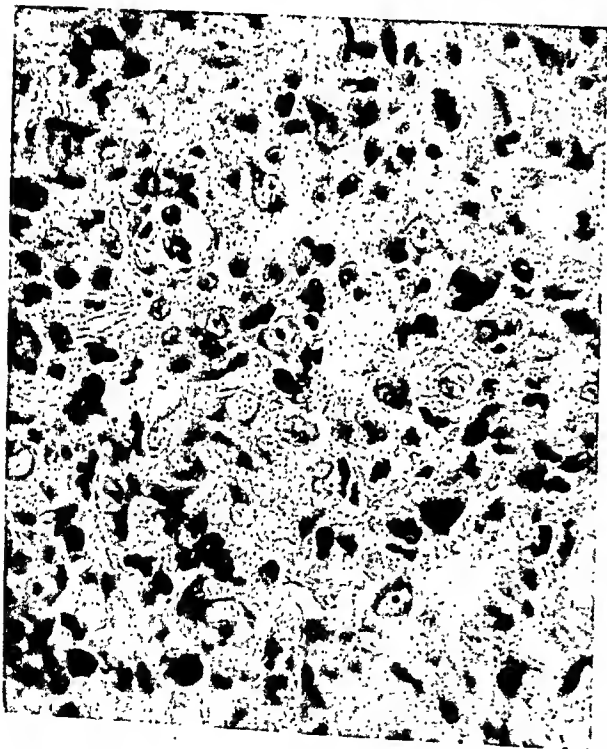
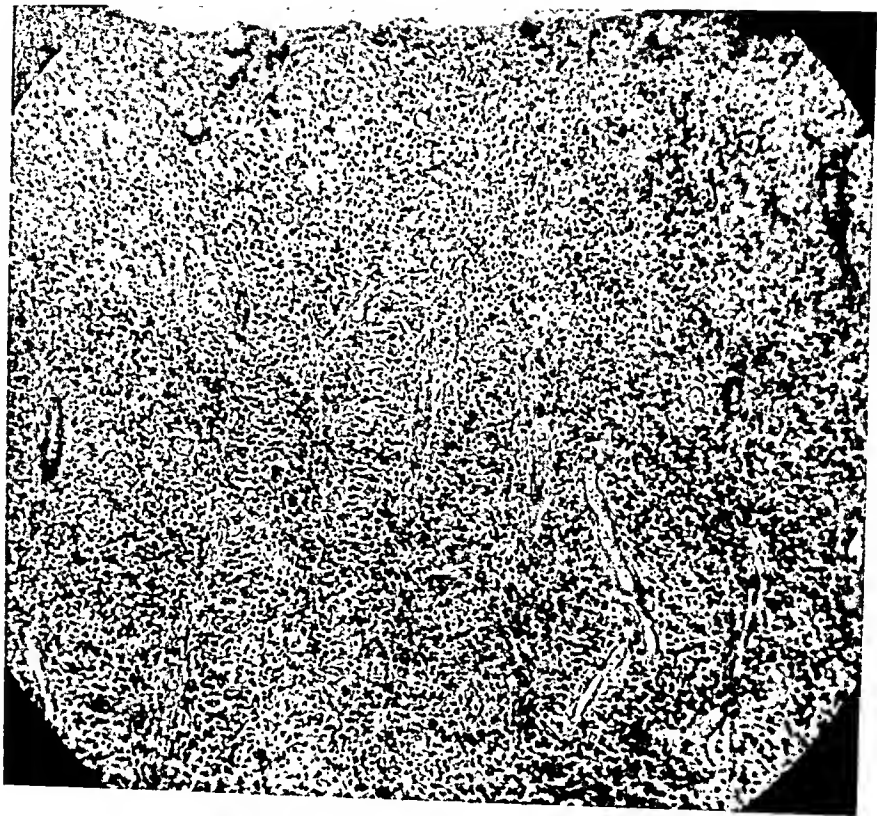
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DESCRIPTION OF PLATES

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## PLATE 88

- FIG. 1. The general pattern of the lesion. The shallow ulcerated surface zone is followed by the mid-zone and by the deep layer. In the mid-zone there are numerous blood vessels with some palisading. Some vessels show degeneration of their walls. Phloxine-methylene blue stain.  $\times 80$ .
- FIG. 2. Endothelial cells in varying stages of proliferation in the mid-zone. Endothelial cells outnumber all others, most of which are polymorphonuclear leukocytes. Phloxine-methylene blue stain.  $\times 600$ .
- FIG. 3. The transition of the mid-zone into the base of the ulcer where polymorphonuclear leukocytes predominate. The small blood vessel in the center shows degeneration of the wall and early thrombus formation. Below and to the right of the vessel is a small group of endothelial cells undergoing necrosis. Phloxine-methylene blue stain.  $\times 550$ .



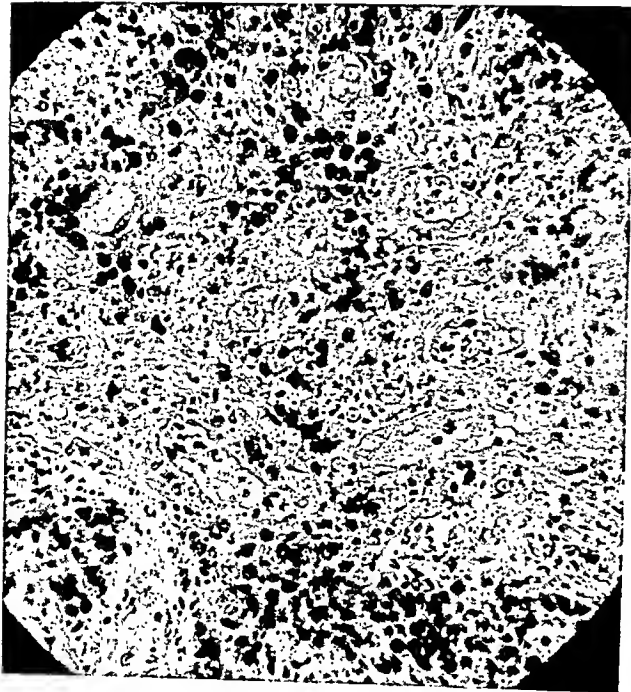
Sheldon and Heyman

Diagnosis of Chancroid by Biopsy

PLATE 89

- FIG. 4. The numerous blood vessels in the mid-zone of the lesion showing proliferation of their endothelial lining. Phloxine-methylene blue stain.  $\times 230$ .
- FIG. 5. Another small artery of the mid-zone near the base of the ulcer with leukocytic infiltration of the wall and beginning fibrinoid degeneration. Phloxine-methylene blue stain.  $\times 550$ .
- FIG. 6. The mid-zone in a healing lesion of 28 days' duration. Of note are the thick walls of the blood vessels, the increase in interstitial connective tissue, and the lymphoid and plasma cell infiltration. Phloxine-methylene blue stain.  $\times 200$ .
- FIG. 7. Higher magnification of the lesion shown in Figure 6. Some vessels have become atrophic and consist only of small whorls of connective tissue. Phloxine-methylene blue stain.  $\times 420$ .

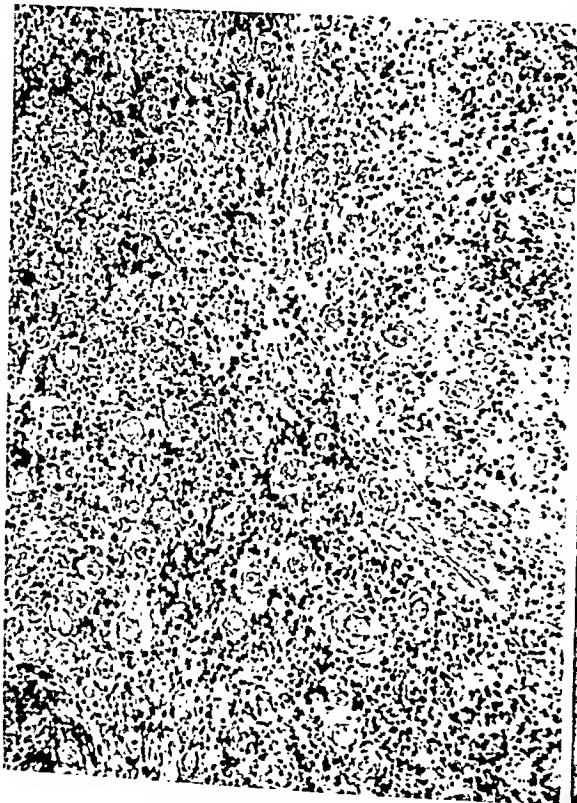
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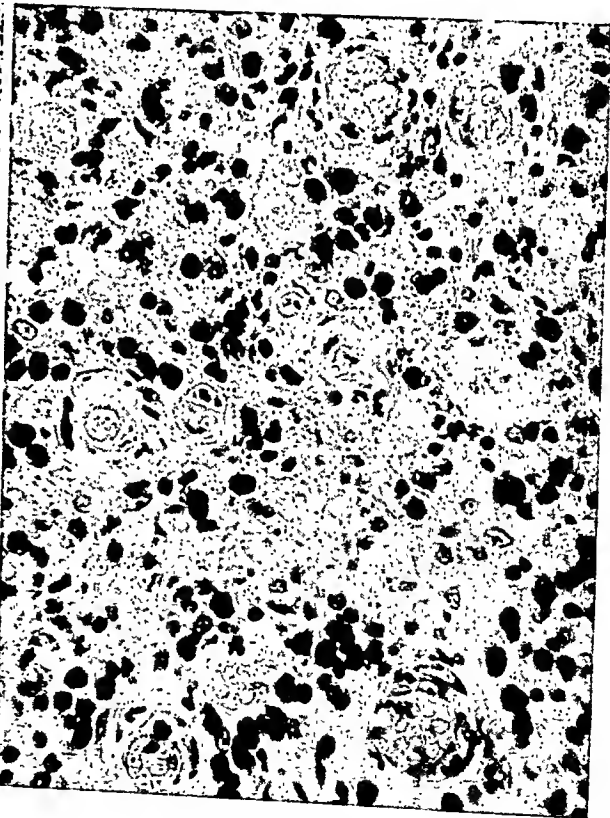
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# THE PROBLEM OF HUMAN TOXOPLASMA CARRIERS \*

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Newborn children can exhibit the fullfledged picture of toxoplasmic encephalitis while the mother is healthy. Therefore, it stands to reason that the mother must have harbored the parasite at least temporarily. An editorial in the *Journal of the American Medical Association*,<sup>1</sup> closing with the words "Are there carriers of Toxoplasma?", put a question every worker in the field must have asked himself, and the literature contains pertinent remarks.

Two observations, made in the course of routine autopsy work, illustrate the difficulty of the problem, and they may stimulate the interest of pathologists.

## FIRST OBSERVATION

The first observation was made upon a 14-year-old white boy who had succumbed to sub-chronic yellow atrophy of the liver. His previous history was noncontributory. Five months before admission jaundice was ushered in by an attack of abdominal pain. The stools were light in color. For the ensuing 2 months the jaundice varied in intensity but never disappeared. About 2 months before admission a physician noted swelling of the abdomen and enlargement of the liver and spleen. Jaundice remained; there was neither pain nor itching; the urine is said not to have been dark. Appetite remained good. During the week before admission the boy vomited a few times. Legs and ankles began to swell. On admission he was deeply jaundiced and drowsy. The abdomen was distended, doughy, and no masses could be palpated. The tonsils were large and the nostrils contained dry blood. The patient declined rapidly. Jaundice deepened; the temperature rose to 105° F., and he became disoriented. There was bleeding from the right tonsil and bloody vomitus. An agglutination test for Weil's disease was negative. The clinical diagnosis was subacute atrophy of the liver (Dr. I. W. Held). Death occurred on the tenth hospital day. This boy had lived on Long Island, near the seashore. There were rats about, but he did not recall having been bitten.

At *autopsy*, severe jaundice was noted. The abdominal cavity contained 2000 cc. of thin yellow fluid with some flakes of fibrin. The spleen was large and soft (440 gm.). The liver (1290 gm.) was nodular; the nodules measured up to 1 cm. and were bright yellow with hemorrhagic areas. Between the nodules the liver tissue was sunken, yellowish pink, without markings. There were partly ruptured varices above the cardia, and some lung areas were filled with blood. The third portion of duodenum and adjoining loops of jejunum were phlegmonous. The skull could not be opened.

Microscopically, two-fifths of the liver sections consisted of islands of regeneration, the remainder being connective tissue with the usual

\* Received for publication, July 7, 1945.



trabecular and pseudotubular epithelial structures. Necrosis and hemorrhage were not conspicuous. No microorganisms could be demonstrated in liver or spleen. Levaditi stain was negative for organisms. Hemolytic streptococci were cultured from a parapancreatic lymph node. The anatomical diagnoses were: Sub-chronic yellow atrophy of liver, with beginning cirrhosis; ruptured esophageal varices; aspiration of blood; phlegmon of duodenum and jejunum; swelling of spleen.

In the routine section of myocardium, under low magnification, a blue dot caught the eye. It was trapezoidal in shape, the base measuring  $48\ \mu$ , the upper horizontal and the sides,  $20\ \mu$ . This mass was situated in a myocardial fiber which, correspondingly, was thickened to at least twice its diameter. Otherwise it did not differ from surrounding fibers, and there was no tissue reaction (Fig. 1). This mass consisted of countless, closely packed, very small, irregularly round and ovoid appearing particles (Figs. 2 and 3). Between the particles one did not see the red of the muscle fiber but an indefinite purplish pink. The whole structure had a fairly distinct outline, and did not occupy the whole thickness of the myocardial fiber. In the same section, 1 cm. away, a similar structure was found (Fig. 4). Its surroundings also were entirely normal. Its shape was less regular, somewhat that of a triangle, the base measuring  $45\ \mu$ , the sides  $32$  and  $30\ \mu$ . The granules in this structure stained a little darker with hematoxylin. They were round or round-ovoid. Since they obviously were distributed irregularly and since no long forms were found, one may assume that they all were either spherical or round-ovoid. The average diameter of each granule was  $1\ \mu$  (object micrometer, oil immersion). The granules were not homogeneous; they contained dark-staining and light-staining areas. On changing the focus one gained the impression that the dark-staining areas were more peripheral. (This subdivision of the particles into cytoplasm and chromatin is not brought out in the photographs.) No further parasites were found in numerous sections from myocardium and other organs, especially spleen, lymph nodes, and liver. The brain, as mentioned, was not available.

The slide and the photographs were submitted to seven specialists. The verdict of two of them was "most likely *Toxoplasma*." The third one first refused to make a diagnosis but joined in after others had favored *Toxoplasma*. One parasitologist, on seeing the photographs, thought of *Toxoplasma* first; he included *Sarcosporidia* and *Trypanosoma cruzi* for differential diagnosis. From the slide he diagnosed sarcosporidiosis. The next one diagnosed either *Trypanosoma cruzi* or *Leishmania donovani*; he considered the particles too small for Toxo-

plasma, and he felt sure they were neither Sarcosporidia nor Histoplasma. Another, who saw only the photographs, wrote "Toxoplasma or Sarcosporidia," and the last one said, "nothing which is incompatible with Toxoplasma" (photographs only seen).

### *Comment on First Observation*

Considering the divergent opinions of the experts, it seems futile to compare these findings with numerous pictures from the literature. The similarity of my Figures 2 and 3 with Figures 1 and 2 in the paper by Kean and Grocott<sup>2</sup> will strike the readers of this *Journal*. Dr. Kean and I had seen the photomicrographs of each others cases in 1944. There is a similarity also with the photographs labelled sarcosporidiosis in the paper by Gilmore, Kean, and Posey.<sup>3</sup> The reasons given by these authors for calling the organisms Sarcosporidia might be applied—in a certain degree—to Figures 1 and 2. Only one of the specialists thought that the organisms had something to do with the clinical course; he diagnosed Leishmania and considered the whole case kala-azar. It is for this reason that the history of the case is given in some detail. No parasites could be found, however, in other organs.

### SECOND OBSERVATION

The structure pictured in Figures 5 and 6 was found in the routine section of myocardium of a 66-year-old woman. This patient died of peritonitis following a two-stage operation for intussusception, caused by a large, partly carcinomatous polyp. There was no tissue reaction that could be ascribed to the presence of this microorganism. The remainder of the autopsy findings were noncontributory. A detailed description seems unnecessary. It might be useful to compare Figure 5 with Figure 432 in Wenyon's Protozoology (1926).<sup>4</sup> Figure 432 is designated as Toxoplasma.

This slide (or the photographs) were submitted to five specialists. Their opinions were as follows:

1. In all probability, Toxoplasma.
2. No opinion; has seen it once in the human brain.
3. No opinion; has seen it in the brain.
4. Contaminant from water or a similar source; has seen it in brain sections on several occasions.
5. Toxoplasma.

The fact that such organisms have been seen in the brain, and now once in the myocardium, but not in other organs, in my opinion does not speak in favor of a contaminant.

## SUMMARY

1. In two autopsies protozoon-like structures were found in the myocardium. There was no tissue reaction.
2. It is difficult, if not impossible, to classify such isolated findings. *Toxoplasma* and *Sarcosporidia* are the most likely diagnoses in the first observation. There is only a possibility that we may deal with *Toxoplasma* in the second observation.
3. The study and discussion of such occurrences are important because we must find the human carriers of *Toxoplasma*.

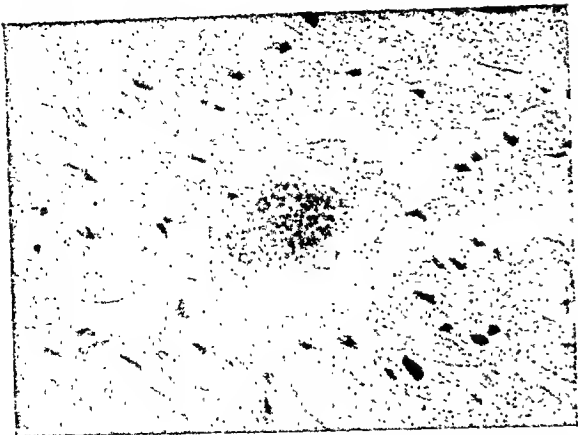
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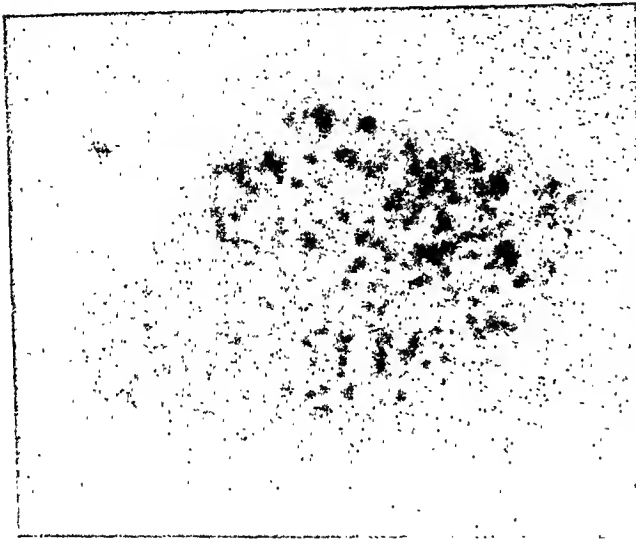
## DESCRIPTION OF PLATE

## PLATE 90

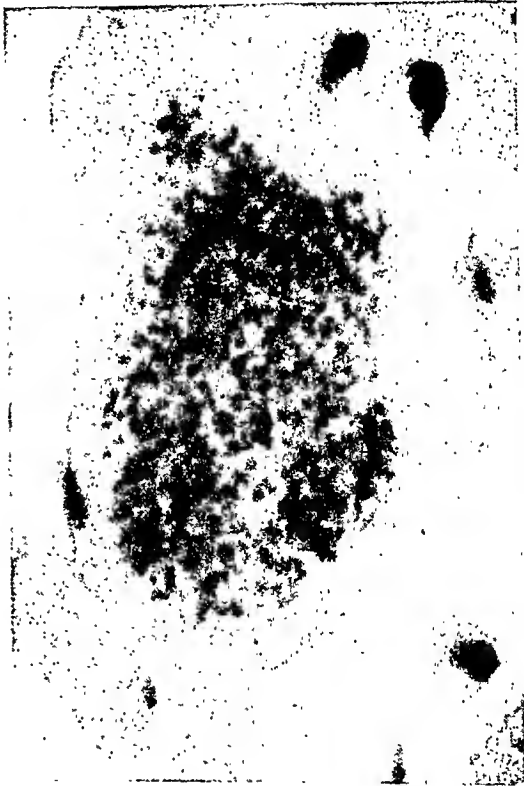
- FIG. 1. Parasite, perhaps *Toxoplasma*, in the myocardium of a 14-year-old boy. No tissue reaction. Hematoxylin and eosin stain.  $\times 360$ .
- FIG. 2. Higher magnification of Figure 1.  $\times 960$ .
- FIG. 3. Higher magnification of Figures 1 and 2.  $\times 1450$ . The parasite does not occupy the whole thickness of the muscle fiber.
- FIG. 4. Another parasite from the same section as shown in Figures 1, 2, and 3.  $\times 1450$ .
- FIG. 5. Nucleated, ovoid organisms, one end pointed, radially arranged. From the myocardium of a 66-year-old woman. No tissue reaction. Hematoxylin and eosin stain.  $\times 600$ .
- FIG. 6. Higher magnification of the smallest rosette from Figure 5.  $\times 1450$ .



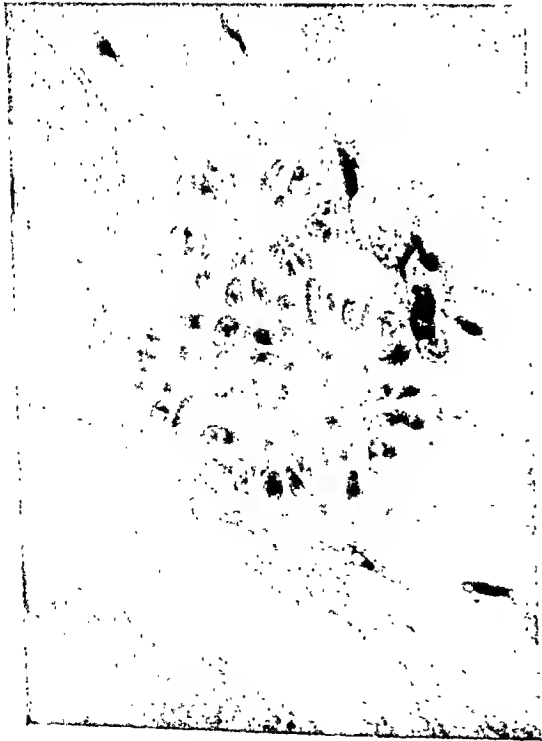
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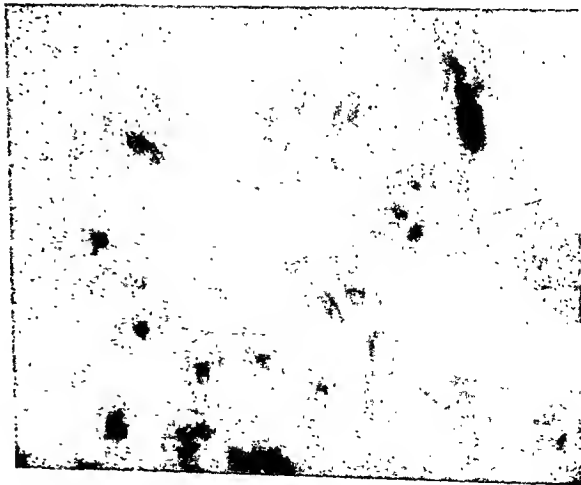
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Human Toxoplasma Carriers



# THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XXII

MAY, 1946

NUMBER 3

## ODONTOGENIC TUMORS

### A CLASSIFICATION BASED ON OBSERVATIONS OF THE EPITHELIAL, MESENCHYMAL, AND MIXED VARIETIES \*

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## INTRODUCTION

Odontogenic tumors, derived from dental or potential dental tissue, are common and have been discussed extensively in the literature. They have been classified as ameloblastomas, dentinomas, cementomas, and odontomas, each having been regarded as a distinct entity. There have been a few cases described in which one type arose in conjunction with another, but no report has suggested the influence of one tissue upon another in the pathogenesis of these tumors. The purpose of this report is three-fold: (1) to formulate a classification of odontogenic tumors in light of the large amount of material studied; (2) to describe their structure, especially that of the mixed group; and (3) to illustrate the inductive effects of one tissue on another in the production of odontogenic mixed tumors.

The material used in this study comprises 64 cases of odontogenic tumor selected from the files of the Registry of Dental and Oral Pathology and from the collection of one of us (K.H.T.). Twenty-six are chiefly epithelial tumors (Table I); 14, mesenchymal tumors (Table II), and 24, odontogenic mixed tumors (Table III).

## EMBRYOLOGY AND HISTOLOGY OF THE TOOTH GERM

Since this study is concerned with tumors derived from dental tissues, a brief review of the normal embryology of the tooth seems essential. The precursor of the tooth bud appears as a thickening of the oral epithelium during the sixth week of embryonic life. This thickening is the primordium of the ectodermal portion of the tooth known as the dental lamina. The tooth bud, in the form of an invagination of the thickening, begins to take shape and the surrounding connective

\* Received for publication, June 11, 1945.

tissue differentiates into the dental papilla. Text-Figure 1 is a drawing of a tooth germ during the sixth month. The dental lamina is recognized at DL, while the permanent tooth bud is seen at A. At this stage the cells which form the tissues of the tooth have differentiated into the outer epithelial layer, the stellate reticulum, the stratum intermedium, the ameloblastic layer, the odontoblastic layer, and the dental papilla. Between the ameloblastic and odontoblastic layers are enamel and dentin. . . .



Text-Fig. 1. A diagrammatic drawing of a tooth germ in the sixth month of embryonic life. Enamel and dentin have been laid down. DL, dental lamina; A, anlage of permanent tooth; OE, outer enamel epithelium; SR, stellate reticulum; SI, stratum intermedium; AM, ameloblastic layer; E, enamel; D, dentin; OL, odontoblastic layer; P, dental pulp.

#### CLASSIFICATION

We are in accord with the classification of odontogenic tumors based on structure; however, certain terms are used in this paper with modifications in meaning. "Odontogenic fibroma" is used to indicate the

soft mesenchymal tumor, while "dentinoma" is reserved for the pure mesenchymal tumor composed of islands of dentin in a connective tissue stroma. "Odontoma" is regarded as a mixed tumor made up of epithelial and mesenchymal elements and is spoken of as "odontogenic mixed tumor." Three types are recognized.

The following classification is the one adopted by us:

- I. Epithelial tumors
  1. Adamantoblastoma
  2. Enameloma
- II. Mesenchymal tumors
  1. Odontogenic fibroma
  2. Dentinoma
  3. Cementoma
- III. Odontogenic mixed tumors (odontomas)
  1. Soft odontoma—epithelium and mesoderm
  2. Soft and calcified odontoma—adamantoblastoma arising in conjunction with a forming or completely formed odontoma; all sorts of histologic variations due to the inductive effects of one tissue on another
  3. Completely formed odontoma with enamel, dentin, pulp, cementum, periodontal membrane
    - a. Compound (many small teeth)
    - b. Complex (irregular tooth structure)

Tumors may arise from either odontogenic epithelium or mesenchyma and may be either soft or calcified. The soft epithelial tumor is commonly called adamantoblastoma; the calcified, enameloma. The mesenchymal odontogenic tumor arises as a fibroma: in the calcified stage, if dentin is produced it is called dentinoma; if cementum, cementoma. Tumors may also originate from both the odontogenic epithelium and mesenchyma. These are known as odontomas or odontogenic mixed tumors and may belong to any one of three groups: (1) soft odontomas, (2) soft and calcified odontomas, and (3) completely calcified odontomas.

### *Epithelial Tumors*

*Adamantoblastoma.* The adamantoblastoma is an epithelial odontogenic tumor in which the cells may differentiate to a variety of forms depending to some extent on the development of the epithelium at the time when tumor formation begins. Solid and cystic types are distinguished, both being of a slowly growing, benign nature, to judge from clinical observation. The frequency of recurrence after operation is due rather to the difficulty of removing the entire lesion by conservative operation than to any malignancy of the tumor; however, several cases recorded<sup>1, 2</sup> indicate that it can be malignant.

Microscopically, the tumor may appear solid, cystic, or a combination of both. If solid, it is composed of cords or strands of epithelium growing in a connective tissue stroma, or occasionally almost without



TABLE I  
*Adamantoblastoma*

Case no.	A.I.P.* accession	Sex	Race	Age	History and symptoms	Location	Roentgenologic findings
1	69715	M	W	54	Noticed nodule 6 years previously, operated 5 years previously, swelling	Mandible	Cystic destruction from symphysis to angle of mandible
2	51505	M	W	24	Pain	Mandible	Dentigerous cyst involving 3rd molar and extending into ramus of mandible
3	75597	M	W	52	Oral-antral sinus, 25 years	Maxillary sinus	Opacity of sinus
4	72782	F	W	27	Swelling	Mandible	Cyst
5	75464	M	W	42	Large movable mass in tuberosity	Maxilla	
6	85184	M	W	36	Removal of dentigerous cyst 4 years previously	Mandible	Cyst in 3rd molar region, no tooth present
7	87202	M	C	21		Mandible	Cyst in 3rd molar region
8	86153	M	W	26	Removal of dentigerous cyst 4 years previously	Maxilla	Cystic lesion from central and incisor region to molar region
9	95817	F	C	37	24 operations performed during a period of 15 years (metastasized to lungs)	Mandible	Cystic lesion involving the body and ramus of mandible
10	108545	M	W	19	During a scuffle received a blow—pain and swelling	Mandible	Dentigerous cyst involving crown of 3rd molar, dentigerous cyst on opposite side
11	117002	M	W	21	Tenderness and swelling at angle of mandible of 48 hours' duration	Mandible	Dentigerous cyst—six other dentigerous cysts present in mandible and maxilla
12	117378	M	W	25	First noticed swelling 6 years ago—area incised and drained	Mandible	Multilocular lesion—no teeth present
13	120765	M	W	25	Swelling of 6 months' duration—no pain	Mandible	Multilocular lesion involving entire body of mandible and extending to inferior border
14	111372	F	W	2½		Mandible	Monocystic lesion
15	101010	M	W	36	Tumor first recognized 7 years previously	Mandible	Cyst in body of mandible
16	103102	M	W	25	Slight swelling, with mild dull pain, 2 years' duration	Mandible	Cyst in region of 2nd premolar and 1st molar

TABLE I (Continued)

Case no.	A.I.P.* accession	Sex	Race	Age	History and symptoms	Location	Roentgenologic findings
17	109169	M	W	17	Previous operation, 3 teeth removed	Mandible	Multilocular cyst beneath 3rd molar
18	103313	M	W	23	Operation 5 years previously	Mandible	Multilocular cyst from canine region extending posteriorly into ramus—no teeth present
19	100372	M	W	31	2nd molar removed 4 years previously—roughening of gum 1½ years previously	Mandible	Dentigerous cyst
20	94818	F	W	21	Swelling for 4 years, two operations	Mandible	Multilocular cyst
21	86845	M	W	28	Swelling	Mandible	Follicular cyst in 2nd and 3rd molar region
22	78638	M	C	25	Drainage of purulent exudate from area	Mandible	Dentigerous cyst involving crown of 3rd molar
23	66686	M	W			Mandible	
24	65091	F	W	60	3rd molar extracted, resulting in fistula into antrum	Maxilla	Radiolucent area in 3rd molar region
25	118679	M	W	32	Painless swelling, 10 years' duration	Mandible	Dentigerous cyst involving crown of canine with radiopaque masses in cystic portion

*Enameloma*

26	H.M.G.	M	W	65	No symptoms	Mandible	Found on examination of autopsy specimen
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\* Army Institute of Pathology.

stroma. The epithelial cords, which may form a network or take on a papillary structure, may resemble the anlage given off from the dental lamina and have a similar tendency to form small buds comparable with the earliest stage of the enamel organ. In the central portion of the cord or lobule the epithelium appears stellate, like the stellate reticulum of the enamel organ; in the periphery the cells are cuboidal to cylindrical.

The cystic type is characterized by lobules or strands of adamantine epithelium with a peripheral layer of cylindrical cells which, in structure, approach ameloblasts. These cells lie on a basement membrane. The central portion consists of stellate to squamous epithelium undergoing cystic degeneration. Pressure of cystic fluid causes these spaces

to enlarge; they often fuse and communicate with one another. Kronfeld<sup>3</sup> believed this kind of adamantoblastoma to be the result of progressive cyst formation in the solid type. He stated that the primary factor in the formation of cystic adamantoblastoma was degeneration of the stellate reticulum of the tumor, a concept supported by Robinson<sup>4</sup> and by data from this study.

The calcifying adamantoblastoma is rare. Kronfeld<sup>3</sup> stated that calcification was sometimes found, but never true enamel; and many other investigators have substantiated the fact that no enamel was produced in adamantoblastomas. In every case in our series the enamel-forming function was lacking, although the ameloblastic stage of development had been reached by groups of cells. Polarization of the ameloblasts was not encountered in adamantoblastomas but was seen in odontogenic mixed tumors.

The adenoid adamantoblastoma is the least common and is composed of small epithelial cells arranged in acini which usually contain a mucoid substance. The oral epithelium has potentialities of forming glandular as well as dental structures and, since the components of the enamel organ are derived from the epithelium of the oral cavity, cells may be present which have the ability to differentiate into adenomatous structures. Tissue of this kind composed one of the adamantoblastomas studied and was present in three of the odontogenic mixed tumors. The acinar arrangement was characteristic and the mucoid material filling the acini seemed to attract calcium salts. The globular pattern of the calcification suggested calcospherites.

The *enameloma* is a small tumor, sometimes situated between the roots of two teeth, but more frequently attached at the bifurcation of the roots of molars and premolars and at the cervical margin of single-rooted teeth. Sometimes spoken of as enamel drops or enamel pearls, enamelomas should be differentiated from small supernumerary teeth containing dentin and pulp. Microscopically, the enamel which appears as a space in the decalcified section is covered by a layer of atrophied epithelial cells, occasionally by a layer of cementum.

### *Mesenchymal Odontogenic Tumors*

Odontogenic fibroma arises from the mesenchymal portion of the tooth germ, that is, from the embryonic tissue of the dental papilla or dental follicle, and, later, from the periodontal membrane. It may, therefore, occur in the coronal or apical regions of the tooth; if in the latter, it may be attached to the tooth and is often mistaken for a granuloma or an odontogenic cyst. One of us (K.H.T.)<sup>5</sup> has reported a case in which roentgenograms showed the tumor as a cystic

TABLE II  
Odontogenic Fibroma

Case no.	A.I.P.* accession	Sex	Race	Age	History and symptoms	Location	Roentgenologic findings
27	M J	F	W	17	Toothache—swelling of gingiva	Mandible	Erupting 3rd molar
28	O P					Mandible	Large cystic area in periapical region of an impacted 3rd molar

*Cementoma*

29	80340	F	W	32	Tooth extracted and cementoma removed—recurred 5 years later	Mandible	Large cystic area in region of 2nd premolar, radiopaque mass in center
30	83048	M	W	32	Pain, lower molar	Mandible	Large radiopaque mass overlying root of 1st molar
31	95725	F	W		Routine roentgenologic examination	Mandible	Radiopaque mass lying between the roots of the 1st molar, surrounded by a translucent area
32	108526	M	W	43	Routine roentgenologic examination	Mandible	Radiopaque mass in the periapical region of the central incisor
33	121058	F	W		Routine roentgenologic examination	Mandible	Radiopaque mass rimmed by a translucent area in edentulous area, approximately region of 1st molar
34	121256	M	W	38	Routine roentgenologic examination; L13 and 14 had been removed 5 years previously	Mandible	Region of L14, edentulous, large cystic area in which was a round, radiopaque mass
35	115924	F	C	44	Hard mass protruded through membrane of mandible under lower denture	Mandible	Large, irregular, radiopaque mass, separated from the mandible by a thin translucent area
36	116894	F	C	45	Large external swelling with fluctuation	Mandible	Mottled area in edentulous mandible resembling sequestration of bone
37	111495	M	W	34	Routine roentgenologic examination	Mandible	Large radiopaque mass seemingly attached to the roots of the 3rd molar
38	110636	M	W	26	Routine roentgenologic examination (multiple cementoma)	Mandible	Radiolucent area surrounding each tooth from canine to canine, in some of which radiopaque masses were seen

TABLE II (Continued)

*Dentinoma*

Case no.	A.I.P.* accession	Sex	Race	Age	History and symptoms	Location	Roentgenologic findings
39	134322	M	W	6	Difficulty in breathing, mucopurulent discharge for 2 years	Maxilla and maxillary sinus	Radiolucent area

*Mixed Dentinoma and Cementoma*

40	131700	F	W	26	Pain	Mandible	Large, irregular, radiopaque mass in periapical region of central necrosis
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\* Army Institute of Pathology.

lesion enveloping the root of a partially erupted mandibular third molar. The tumor consisted of a solid mass composed of embryonal connective tissue containing small spindle-shaped cells. No inflammatory infiltration was present. In the fibrous stage it is impossible to determine the outcome of the lesion since it may form either a dentinoma or a cementoma. Dentinoma is usual in the coronal region; cementoma, in the apical region.

The *dentinoma* in the pure form is rare; however, the same process may be found in a few of the odontogenic mixed tumors. The *dentinoma*, composed of odontoblastic tissue, is made up of denticles or islands of irregularly formed dentin in a stroma consisting of connective tissue with enmeshed cells of various shapes, spindle and round being more common. The dentin contains fewer and more tortuous tubules than normal and resembles secondary dentin laid down in the pulp in advance of caries. Some of the dentin encloses living cells to appear as if it had been secreted around odontoblasts. Certain of the interstitial cells closely resemble bone corpuscles and this type of tissue may be referred to as osteodentin.

The *cementoma* was described by one of us (K.H.T.),<sup>6</sup> who pointed out that it was a secondary formation, the by-product of a soft tissue tumor, the cementoblastoma (odontogenic fibroma). In the growing stage the cellular tissue is predominant, diminishing in proportion as cementum is formed. When the cellular elements have exhausted their activity, they remain as a thin connective tissue capsule around the calcified tumor; thus the histologic picture varies with the stage through which the tumor is passing. The cementoblastoma may produce cementicles which become fused, or trabeculae may be laid down

in lamellar fashion. It is difficult to distinguish cementum from bone, especially when there is cementoblastic activity as well as cementoblastic deposition. Cementum, however, has a fibrillar matrix, a more irregular lamination, and fewer inclosed cells than bone.

*Tumors Originating from Both Odontogenic Epithelium  
and Mesenchyma*

The odontogenic mixed tumor is composed of the epithelial and mesenchymal portions of the tooth germ with the potentialities of those structures to form enamel, dentin, cementum, pulp, and periodontal tissue. Both soft and calcified types are encountered.

*Soft Odontoma.* It has been observed that the stroma is often prominent in the solid type of ameloblastoma. Bauer<sup>7</sup> recognized that in cystic ameloblastomas there was usually an insignificant increase of the connective tissue, while in the more solid tumors the stroma was greatly increased and played an important rôle. Thoma<sup>5</sup> described soft odontoma as derived from the mesenchymal part of the tooth germ which may form from either the embryonic mesenchymal tissue or the dental follicle. He believed that soft odontoma might be classified as central fibroma or fibro-odontoma; however, as dental epithelium was present in many of these tumors, the term fibro-adamantinoma might be justified. Nagel<sup>8</sup> pointed out that these soft tumors were easily mistaken for ameloblastomas.

In odontogenic mixed tumors of the soft type, the epithelium is usually arranged in lobules or strands, the peripheral cells of which are more columnar and resemble prefunctional ameloblasts. The central cells are often squamous to spindle-shaped, or assume a stellate-reticular form, like that seen in the enamel organ. Sometimes the epithelial strands suggest buds or the bell-shape of the early enamel organ. The stroma is abundant, at times embryonal, with stellate fibroblasts as seen in the dentinal papilla and young pulp of a tooth. It may resemble more mature connective tissue in which collagen is deposited and numerous blood vessels are present. The stroma may become fibromyxomatous. Occasionally the tumor is composed almost entirely of connective tissue with epithelium a minor component.

*Soft and calcified odontomas* have been divided into two groups: the first characterized by a soft epithelial component, a soft mesenchymal component, and dentin; the second, by a soft epithelial component and enamel, soft mesenchymal component, and both dentin and cementum. The microscopic picture of the first type, as seen in case 44, was similar to that of the soft odontogenic mixed tumor except that dentin was produced. The nests of adamantine tissue were

TABLE III  
Odontogenic Mixed Tumors

Case no.	A.I.P.* accession	Sex	Race	Age	History and symptoms	Location	Röntgenologic findings
41	101059	M	W	29	Painless, slow-growing, hard tumor	Mandible	Multicystic area from bicuspid to molar region and beneath the inferior border, all teeth present
42	61743	M	C	39	Swelling, pain, discharge	Mandible	Multicystic lesion involving the molar region and entire ramus
43	107940	M	W	5		Mandible	Cyst
44	118866	M	W	59	Slight swelling, paresthesia of lip	Mandible	Two large cystic lesions, broken down teeth present but apparently unrelated
45	133773	M	W	16	Swelling—central and lateral incisors tender to touch	Maxilla	Large radiopaque mass in cystic-appearing lesion, all teeth present
46	132671	F	W	35	Painless swelling	Mandible	Radiopaque masses in dentigerous cyst
47	133772	M	W	15	Gradual swelling	Mandible	Dentigerous cyst involving 2nd molar, follicular cyst in 3rd molar area, containing radiopaque masses, 3rd molar absent
48	74998	F	W	9	Failure of teeth to erupt	Mandible	Dentigerous and follicular cysts
49	95083	M	W	22	Tumor of right maxilla removed 2 years previously—fungating bleeding mass at canine fossa	Maxilla	Cystic area in canine region
50	115927	M	C	26	Slight swelling	Mandible	Irregular radiopaque mass in cystic area spreading the roots of the canine and premolar
51	63400	M	W	3	Absence of tooth—area slightly tender to percussion	Mandible	Radiopaque mass in cyst above deciduous tooth
52	127807	M	W	8	Failure of deciduous teeth to erupt	Maxilla	Small rudimentary teeth and irregular radiopaque masses
53	84173	M	W	22	Pain, thought to be due to wisdom tooth erupting	Mandible	Round, smooth, radiopaque mass rimmed by a thin line of decreased density beneath which was 3rd molar
54	121695	M	C	27		Mandible	Dentigerous cyst with odontoma in cyst, 1st molar

TABLE III (continued)

Case no.	A.I.P.* accession	Sex	Race	Age	History and symptoms	Location	Roentgenologic findings
55	32007					Maxilla	Large, irregular, radiopaque mass in the molar region—no regular tooth formation
56	72697	M	W	21	Pain	Mandible	Cyst with radiopaque mass in 3rd molar region
57	88341	M	W			Maxillary sinus	Radiopaque mass in right maxillary sinus
58	105341	M	W	20	Asymptomatic swelling	Mandible	Large, irregular, radiopaque mass occupying the 3rd molar region and part of the ramus, displacing the 3rd molar toward the angle of the mandible
59	105338	M	W	24	Asymptomatic swelling	Mandible	Large, irregular, radiopaque mass in molar region; a molar tooth appeared beneath the mass; no teeth were present before the first premolar
60	40486	M	W	19	Pain	Maxilla	Large, irregular, radiopaque mass in 3rd molar region; the 3rd molar lay superior and anterior to the mass
61	40487					Mandible	Follicular cyst with odontoma
62	61365	F	W	18	Pain	Maxilla	Irregular, radiopaque mass in 2nd molar region—no 3rd molar evident
63	59780	M	W	25	None	Maxilla	Radiopaque mass in 2nd and 3rd molar region
64	134323	M	W	31	Irregularity in eruption of teeth	Maxilla	Irregular, radiopaque formations in region of maxillary and lateral canine—two teeth above mass

\* Army Institute of Pathology.

rimmed by dentin which was irregular in structure as is secondary dentin. The dentinal tubules were fewer and were tortuous in contrast to their regularity in normal dentin. This process could be interpreted as a response of the stroma by forming dentin in the presence of adamantine tissue, a reaction commonly observed in odontogenic mixed tumors.



Perhaps the most interesting of all the odontogenic tumors are those in which all possible phases of dental production are seen. In certain tumors, both fully formed and rudimentary teeth or irregular dental structures are found. Areas of pure adamantoblastoma, of pure dentinoma, and of epithelial tumor in conjunction with dentin production may be encountered as well as tooth buds in all stages. These processes are well illustrated in cases 46, 52, and 45. In case 46, an abortive tooth root, composed of irregularly formed dentin covered by cementum, was adjacent to small rudimentary teeth made up of an epithelial membrane around the enamel spaces. Occasional epithelial sprouts arose from this membrane. In one area the tissue consisted almost entirely of proliferating epithelium which developed in the form of irregular follicles surrounded by a rim of dentin. The epithelium consisted of an outer layer of cylindrical cells and a central portion of squamous cells which tended to form pseudo-pearls. In places, the rim of dentin was absent; in others, dentin formation was beginning. In case 52 the processes were essentially the same, but in addition there were areas of dentin laid down in denticle fashion, epithelium undergoing necrobiosis, and epithelium arranged in acini filled with a colloid-like material. In case 45, filling the cavity of a cyst was a mass of irregular dental structures in a connective tissue stroma. Between these structures were concentric masses of necrobiotic epithelial cells in which focal areas of calcification were noted. Scattered through the collections of dentin and necrobiotic epithelium were aggregates of adamantine cells, in some areas solidly packed, in others assuming a cystic pattern. In the capsule of the cyst, lobules of adamantine tissue and scattered ghost-like epithelial cells were intermingled. Of greatest interest was the formation of a small tooth bud in the capsule. It was composed of layers of epithelium, dentin, an irregular odontoblastic layer, and the dental papilla. The epithelium, however, had not differentiated into ameloblasts although a considerable layer of dentin had been laid down. These cases suggest the inductive effects of one tissue upon another. Epithelial lobules seem to evoke the potentiality of the adjacent stroma, causing dentin to be laid down. Dentin was formed despite the lack of an ameloblastic layer and in several instances in the absence of epithelium.

*Completely formed odontomas* include: (1) compound odontomas which are made up of a large number of more or less rudimentary teeth; and (2) complex odontomas in which the calcified structures bear no resemblance to the anatomic arrangement of the dental tissue. These tumors are encompassed by capsules. The pathogenesis of odontoma is brought out in case 52 (Figs. 51 to 56).

The compound type of odontoma contains a large number of teeth and is usually included in a cyst membrane. The dental epithelium, instead of forming the normal enamel organ, produces many small enamel organs which develop into tooth germs and give rise to small teeth of varying size and shape. Several adjoining tooth germs may take part in the process and produce some normal, and many deformed, teeth. Microscopically, the structure appears normal although the teeth may be irregular and dwarfed, and may be attached to one another by fibrous connective tissue, cementum, or bone.

In complex odontoma, the arrangement of the tissue is not regular and tooth-like since the tissue is in different stages of development. This tumor may be produced by a single tooth germ, which has developed abnormally, derived either from a normal tooth or a supernumerary sprout from the dental lamina. Schour, Massler, and Greep<sup>9</sup> followed the development of a complex odontoma in a group of rats in which there was hereditary anodontia of the incisor tooth. A complex odontoma is a hard, solid, rounded or oval mass, surrounded by a capsule from which it usually can be shelled out with ease. Its surface may be covered with cementum having scattered nodules of enamel. Sometimes unerupted, normally-formed teeth may be fused with the tumor. Microscopically, great variations are found in structure and in the proportion of the different tooth elements. In some, an arrangement like that of normal tooth formation is interrupted by irregularly formed tissue. In others, abnormal arrangement of tooth substance is the predominant feature, with enamel, dentin, and cementum distributed in lamellar or radial fashion. Soft tissue, such as enamel epithelium and dental pulp, may be seen between the calcified layers, the former adjoining the enamel, the latter the dentin. The cementum may be found only at the periphery beneath the epithelial fibrous capsule.

#### INDUCTIVE EFFECTS OF ONE TISSUE ON ANOTHER

The deposition of dentin about epithelial lobules, necrobiosis of the epithelium with resultant calcification, and irregular dental patterns bring up the question of the influence of one tissue upon another. In the intimate tissue mixtures of these tumors, neighboring tissues seem to exert inductive influences upon one another, comparable to those known in embryonic development or in mixed tissue cultures. Structural features indicate that odontogenic mixed tumors are not a fortuitous jumble of independent ingredients or a potpourri of tissues, but that the form they take results from the response of one tissue to another.

An outstanding feature of the adamantoblastoma is the failure of enamel production even though certain cells are morphologically almost identical to those seen in the enamel organ of normal tooth formation. Perhaps this failure may be attributed to the fact that polarization of the neoplastic cells does not occur. It is possible that dentin influences the polarization of these cells prior to enamel production. Early dentin formation was observed in several of the soft odontogenic mixed tumors, although no enamel was laid down. Whether or not this was an intermediate stage and enamel would have formed later cannot be determined.

The relationship of the ameloblast to the odontoblast is of interest as well as of importance in the determination of the type of tissue formed and the pattern developed in odontogenic mixed tumors. Huggins, McCarroll, and Dahlberg,<sup>10</sup> by the method of transplanting developing dental tissues, found that enamel was deposited only on dentin, although dentin could be laid down independently of enamel but only when odontoblasts were present. Glasstone,<sup>11</sup> by growing portions of tooth germs *in vitro*, found that the ameloblastic layer was essential for the formation of odontoblasts. Because of these findings, Sprawson<sup>12</sup> believed that in the formation of adamantoblastomas, enamel is not formed. The cells which make up this tumor are basal cells, not ameloblasts, since odontoblasts do not become differentiated. In our study, it appeared that enamel was not formed where there was an absence of dentin and that dentin formation was independent of ameloblasts. This is shown in Figures 7, 16, and 50 and also in the two dentinomas and in like areas in the odontogenic mixed tumors. It is, however, possible that dentin production was initiated by fibroblastic activity with the resultant bone-like dentin seen in these tumors.

The dentinoma has been considered an odontoma, the predominant tissue being dentin. However, in all dentinomas previously described, enamel and cementum were present. A dentinoma consisting purely of dentin and stroma has not hitherto been reported. This tumor arises from the odontogenic fibroma which may differentiate into either cementoma or dentinoma. In one tumor studied, both tissues were present, which suggested their common origin. The structure of the dentinoma demonstrates that dentin may be produced despite the lack of odontogenic epithelium.

The stroma seen in the adamantoblastoma is easily distinguished from the connective tissue observed in the soft odontogenic mixed tumor. It is usually minimal in quantity and does not play an important rôle in the tumor. In the odontogenic mixed tumor, however,

the connective tissue is usually predominant, the epithelium scanty. In these tumors there is a tendency for the production of dentin in apposition to the epithelium. Surrounding many of the epithelial nests is a clear zone, around others a rimming of dentin of the osteodentin type with no odontoblastic layer. Like bone, the dentin has entrapped cells which appear to be of fibroblastic origin.

One must regard the tumors which arise from epithelium and connective tissue as mixed tumors. In further proof are the cases reported as adamantinosarcomas. Krompecher<sup>13</sup> reported one of the first of these. A solid, primary tumor was found in a 13-year-old boy. The histologic picture was that of spindle-cell or round-cell sarcoma, the epithelial part resembling the ordinary type of adamantoblastoma. Wigdortschink<sup>14</sup> described a similar tumor in a boy, 9 years old, who had a lesion in the mandible which appeared to be a sharply circumscribed cyst. Besides highly differentiated adamantine tissue, it contained very cellular connective tissue of a fibrosarcomatous character.

The odontogenic mixed tumor may arise as either a soft or a calcified tumor; the epithelial and mesenchymal elements varying as to differentiation and proportionate quantity. The soft odontogenic mixed tumor is composed of epithelial and mesenchymal elements, and in those observed the connective tissue predominated. Morphologically, it resembles the dental pulp, the cells being stellate. The hard, calcified, odontogenic mixed tumor has been frequently described in the literature and has been termed odontoma. The frequent occurrence of soft epithelial polyhedral and squamous cells as well as adenomatous structures in this hard tumor was noted; in many instances these cells were of adamantine nature like the cells of pure adamantoblastoma. Occasionally, irregular calcospherites were seen in the epithelial nests.

The combination of soft and calcified odontogenic mixed tumor produces many bizarre pictures, because of the varying proportions and arrangements of the epithelial and mesodermal elements, resulting from splitting, budding, and fusion of the odontoblasts.

#### LIMITATION OF GROWTH

From the study of this group of tumors, it appears that differentiation to a variety of forms may take place, depending to a large extent on the development of the epithelium and mesoderm at the time the neoplasm begins to form. It seems that the uncalcified tumors undergo a quantitative growth with but little qualitative alteration, while the calcified ones undergo a quantitative and a qualitative change, that is, their growth is one with differentiation. Where evolution is chiefly quantitative, the possibility of uncontrolled growth is far greater than

when the evolution is chiefly qualitative, for differentiation tends to lead to the development of the final form. Thus we find that the adamantoblastoma is inclined to grow large and be invasive, while the odontoma appears to grow only to a limited size. The odontogenic mixed tumor has the developmental potentialities of both adamantoblastoma and odontoma, a fact that has received further proof from this series of cases.

### SUMMARY

The odontogenic tumors are classified into three groups: epithelial, mesenchymal, and mixed. The dentinoma, a pure mesenchymal tumor, is composed of connective tissue in which denticles or islands of irregularly formed dentin are present. The odontogenic mixed tumors consist of epithelial and mesodermal elements which are in combination in various proportions and arrangements. Three types are recognized: soft, soft and calcified, and calcified. The soft type has been differentiated from the solid adamantoblastoma.

There is evidence of the inductive influences of one tissue on another in the odontogenic mixed tumors. It is noted that epithelium in these tumors seems to stimulate dentin formation, but that the presence of epithelium is not necessary for the production of dentin. Also, dentin is formed in the presence of epithelial cells not differentiated into ameloblasts. Neoplastic adamantine tissue and enamel-forming ameloblasts have been distinguished. The presence of these two types accounts, in part, for the formation of the soft and calcified odontogenic mixed tumors.

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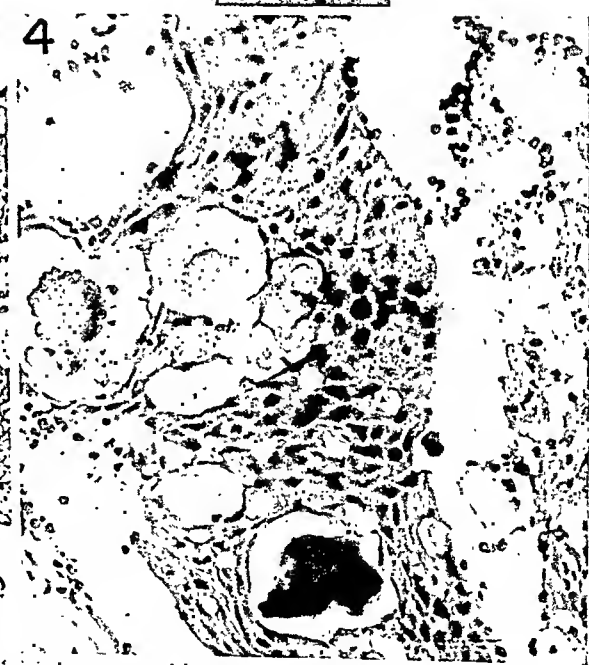
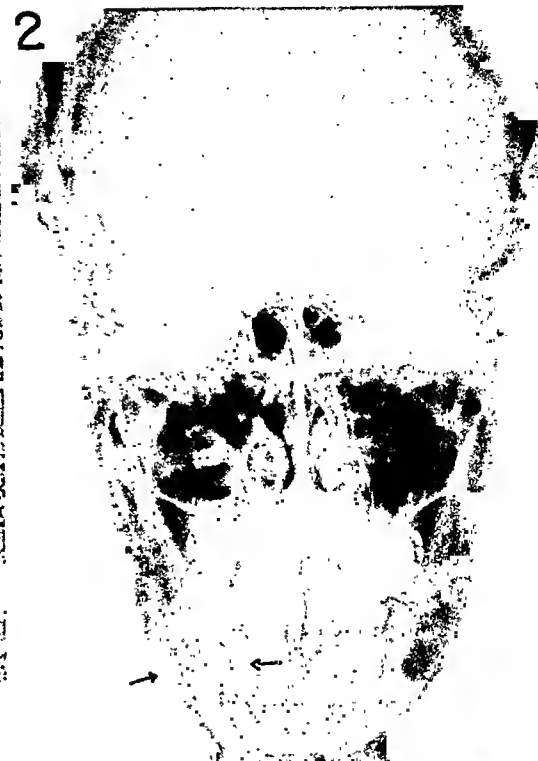
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## DESCRIPTION OF PLATES

### PLATE 91

- FIG. 1. Case 24. *Adamantoblastoma*. A.I.P. Acc. 65091, Neg. '85499. A growth which had caused no pain, was removed from the maxillary region of a woman, 60 years of age. The tumor was made up of sheets of large epithelial cells. Around the edges these cells were palisaded as in the enamel organ (Text-Fig. 1). The central portion consisted, for the most part, of squamous cells, with stellate reticulum of enamel organ type in a few areas. The tumor was almost purely epithelial, with little fibrous stroma evident.  $\times 60$ .
- FIGS. 2 to 5. Case 25. *Adamantoblastoma, adenoid type*. A.I.P. Acc. 118679. A man, 32 years of age, had noted progressive painless swelling on the side of his face for 9 years. Roentgenograms showed a large cystic lesion enveloping the crown of the cuspid tooth (Fig. 2, Neg. 83938). The lesion was removed. Surrounding the root of the cuspid tooth was an overgrowth of brownish gray, relatively soft tissue with numerous, tiny, hard masses loosely grouped together, and other discrete nodules. Microscopically, a rather acellular fibrous tissue stroma surrounded masses of epithelial cells which, for the most part, were polyhedral with prominent intercellular bridges (Fig. 3, Neg. 84047,  $\times 70$ , and Fig. 4, Neg. 84051,  $\times 335$ ). In some instances the cells were oval to columnar and tended to stain lightly, resembling ameloblasts in nuclear detail, position of the nucleus, and intercellular substance (Fig. 5, Neg. 87683,  $\times 450$ ). The nuclei were oval or slightly elongated and of uniform size. The cells were arranged in acini containing a mucoid substance in which there was evidence of focal calcification. Occasionally the cells themselves were calcified, in some areas so heavily that the underlying epithelium was masked.



Thoma and Goldman

Odontogenic Tumors

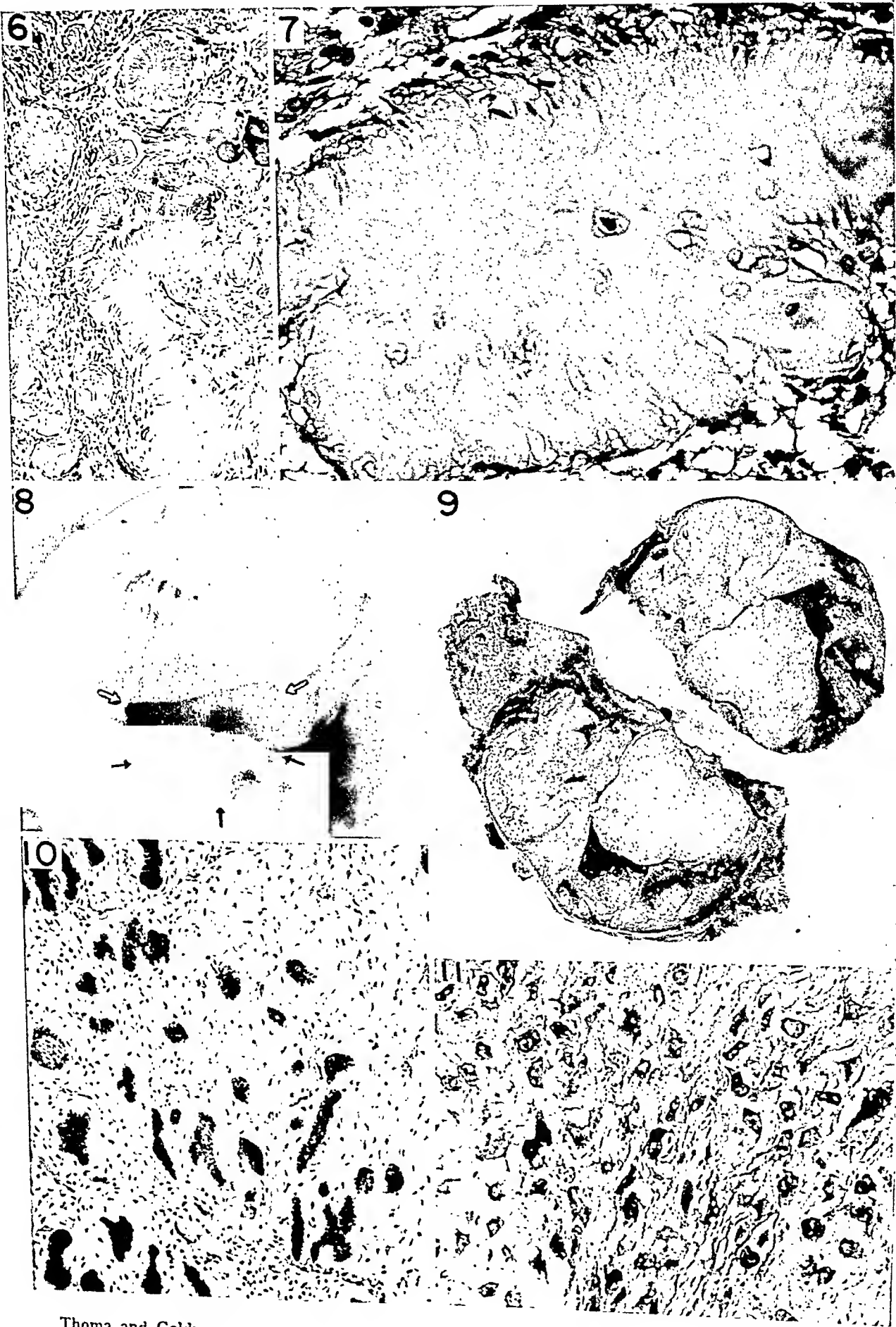


## PLATE 92

FIGS. 6 and 7. Case 39. *Dentinoma*. A.I.P. Acc. 134322. \* The left nostril of a 6-year-old boy was completely obstructed by a firm, moveable mass, which had produced slight asymmetry of the face. Deviation of the septum to the right caused difficulty in breathing through the right nostril. The bone over the maxillary sinus could easily be depressed over an area about an inch in diameter. The first dentition was complete. A rather loose mesenchyma contained numerous round and irregular masses of eosinophilic material recognized as dentin (Fig. 6, Neg. 85488,  $\times 90$ ). This substance was somewhat fibrillar with irregular dentinal tubules; entrapped cells were common (Fig. 7, Neg. 85489,  $\times 370$ ). One part of the tumor was composed exclusively of connective tissue which resembled dental pulp; at the border of the tumor the tissue contained bony spicules which corresponded to the normal bone surrounding the tumor.

FIGS. 8 to 11. Case 41. *Soft odontogenic mixed tumor*. A.I.P. Acc. 101059. A painless mass on the face of a man, 29 years old, was firm except for one fluctuant area anterior to the angle of the mandible. Roentgenologic examination revealed a multicystic area (Fig. 8, Neg. 78852). A large neoplasm had invaded much of the substance of the mandible. The larger portion of the tumor was on the mesial side of the bone, but there were two solid masses within the body of the mandible. The lower border was completely eroded and replaced by a tumor with a bluish surface. It cut with relative ease, was compact, light-yellow, gelatinous, interspersed with a few fine trabeculae, and was surrounded by a thick fibrous capsule (Fig. 9, Neg. 81565). Microscopically, large columns and strands of epithelial cells were embedded in a fibrous matrix. Many epithelial strands were thin and multibranching, winding through contiguous microscopic fields (Fig. 10, Neg. 78576,  $\times 85$ ). In the individual cord the cells in the outermost layer were columnar; those in the center were either squamous or small with clear cytoplasm, giving the tissue a reticulated appearance. Connective tissue of a simple fibrous type was far more abundant in the tumor than epithelial tissue. In many areas, the cells of the stroma were irregularly disposed, their cytoplasm clear, and the nuclei large with prominent nucleoli (Fig. 11, Neg. 85502,  $\times 405$ ).

\* Case sent to K.H.T. by Dr. Louis Berger, Montreal, Canada.



Thoma and Goldman

Odontogenic Tumors

### PLATE 93

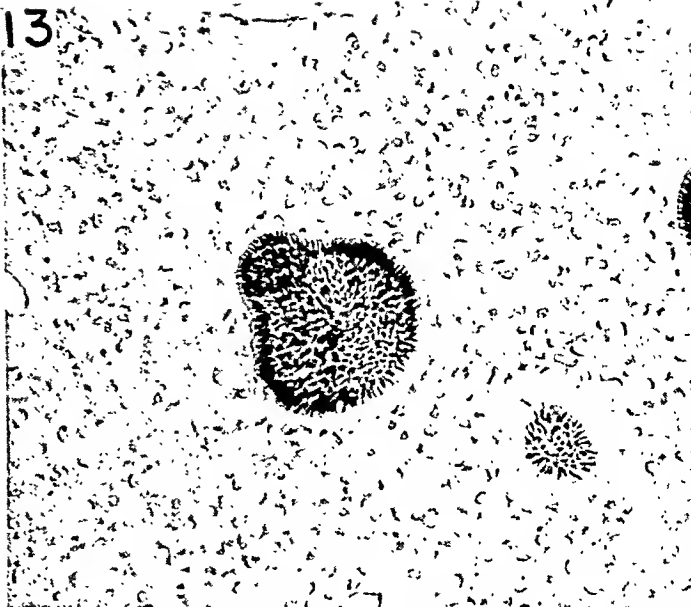
FIGS. 12 and 13. Case 42. *Soft odontogenic mixed tumor*. A.I.P. Acc. 61743. Four years previously a swelling had appeared on the left side of the face of a man, 39 years of age. Two years later limitation of motion of the jaw became apparent. A hard mass extended from the border of the mandible to the temporal region. Roentgenologic examination revealed a large multicystic mass the size of an orange and an unerupted tooth (Fig. 12, Neg. 68186). The left ramus and body of the mandible up to and including the second molar were resected. In several areas the tissue was composed of occasional epithelial strands and lobules in a cellular and edematous connective tissue. The peripheral cells of the lobules were inclined to be columnar while the central cells were squamous and many had assumed the appearance of stellate reticulum (Fig 13, Neg. 85498.  $\times 100$ ). Several areas of the connective tissue portion of the tumor resembled the connective tissue of the dental papilla of tooth formation.

FIGS. 14 to 16. Case 44. *Soft odontogenic mixed tumor with production of dentin*. A.I.P. Acc. 118866. A man, 59 years old, had first observed a slight swelling on the labial aspect of the incisor region 15 years previously. It gradually grew larger. Roentgenograms disclosed two large radiolucent areas in the anterior right portion of the mandible, each associated with a small root (Fig. 14, Neg. 82707). Microscopically, interlacing dense bands of collagen fibers were loosely packed and scattered throughout the loose stroma. Lobules and strands of small, deeply stained cuboidal cells with clear cytoplasm suggested pre-ameloblasts (Fig. 15, Neg. 85500,  $\times 275$ ). Surrounding some of the lobules were rims of eosinophilic material which was identified as dentin (Fig. 16, Neg. 85495,  $\times 305$ ).

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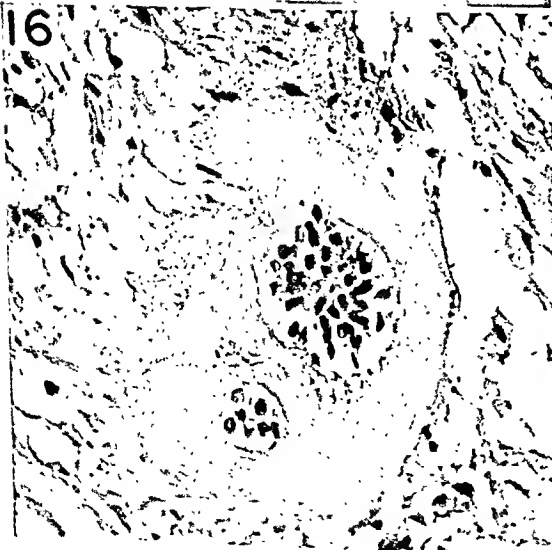
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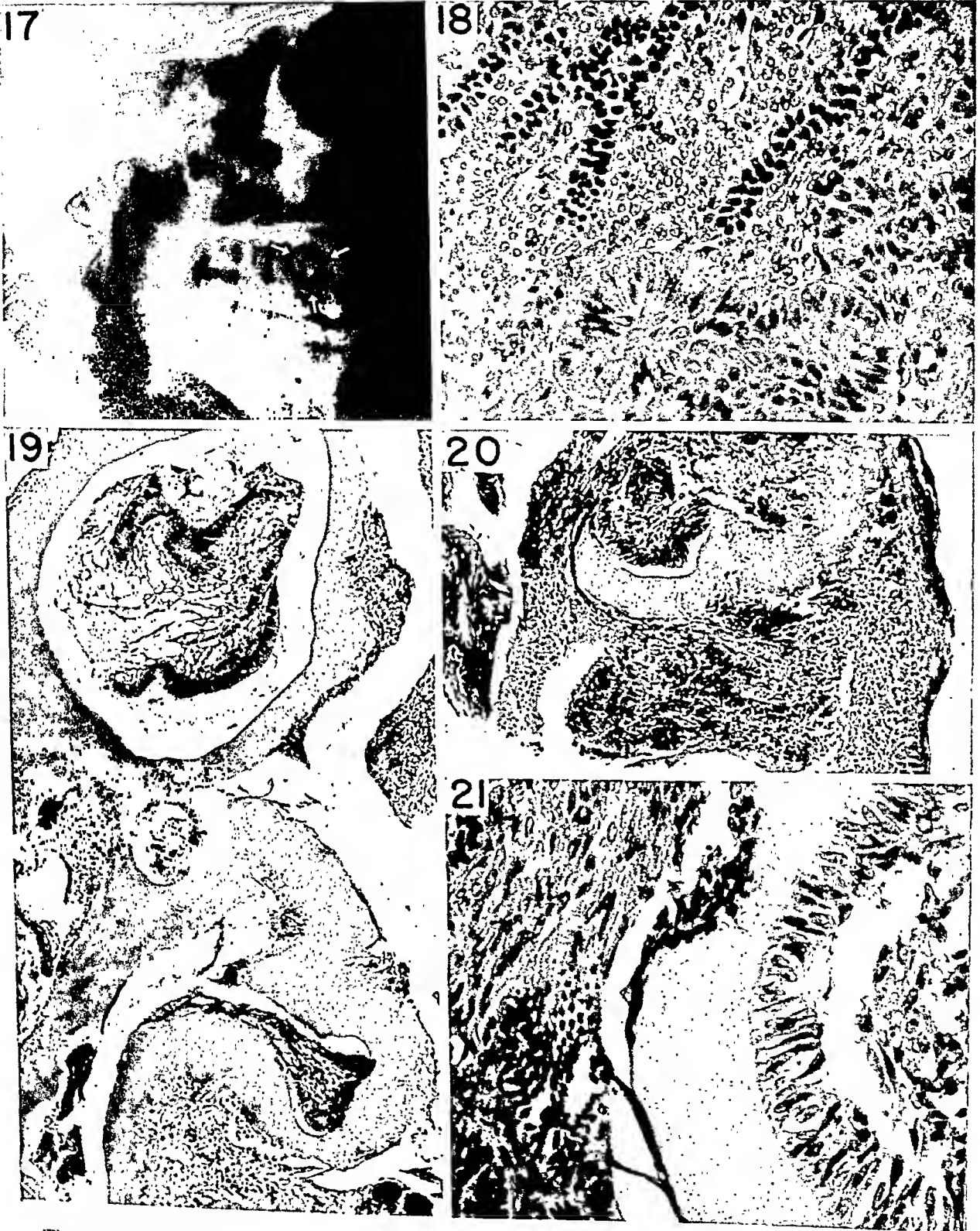


Thoma and Goldman

Odontogenic Tumors

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FIGS. 17 to 21. Case 49. *Odontogenic mixed tumor*. A.I.P. Acc. 95083. Following operation for a tumor of the right maxilla 2 years before, the gum of a man, 22 years old, had gradually increased in size. There was a fungating, bleeding mass in the right canine fossa. Roentgenologic examination disclosed a cystic lesion in the canine region (Fig. 17, Neg. 82113). Fragments of the tumor were hard. The sections varied from proliferating adamantine epithelium (Fig. 18, Neg. 82265,  $\times 270$ ) to abortive dental formations (Fig. 19, Neg. 82264,  $\times 75$ ). Numerous fasciculi of spindle cells were fringed with columnar cells, and in some areas the columnar cells formed radial collars about the spindle cells. One section consisted of masses of adamantine epithelium surrounded by irregular formations of dentin like those seen in the other odontogenic mixed tumors. Rimming the dentin were small, round, deeply staining cells which did not have the structure of odontoblasts, but, nevertheless, seemed to be the cells responsible for the formation of the dentin (Fig. 19, and Fig. 20, Neg. 82263,  $\times 105$ ). Focal calcification of occasional nests of epithelial cells was found. Lining the inner surface of many of the dentin masses were columnar cells morphologically like ameloblasts; the central cells were stellate (Fig. 21, Neg. 82262,  $\times 95$ ).



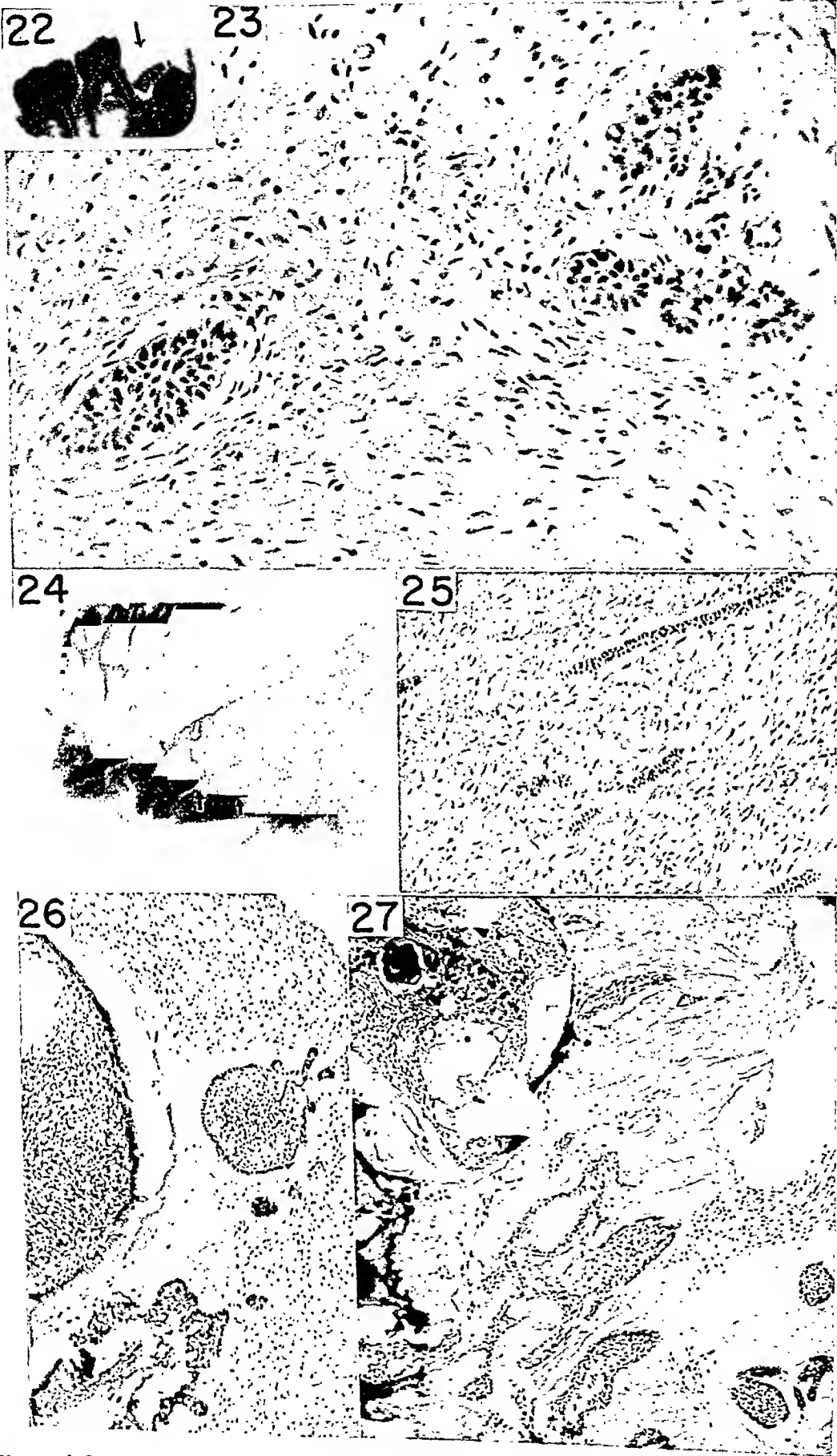
Thoma and Goldman

Odontogenic Tumors

# PLATE 95

FIGS. 22 and 23. Case 51. *Odontogenic mixed tumor*. A.I.P. Acc. 63400. A white boy, 3 years of age, had a swelling over the gum in the lower left deciduous cuspid area where there was no tooth. Roentgenologic examination revealed a cystic lesion which contained small coalesced calcified bodies (Fig. 22, Neg. 78794). The permanent canine could be seen beneath the cystic area. Microscopically, a very loosely formed tissue was composed of stellate and spindle cells such as are seen in the stellate connective tissue of the pulp. Partially covering the section were epithelial cells of columnar type with large, darkly staining nuclei, which appeared like those found in the inner enamel epithelium. Lying in the connective tissue of embryonal type were lobules of epithelial cells (Fig. 23, Neg. 78584,  $\times 360$ ). The calcified bodies were not examined microscopically.

FIGS. 24 to 27. Case 48. *Odontogenic mixed tumor*. A.I.P. Acc. 74998. Delayed eruption of the lower right molar was noted in a girl, 9 years of age. The roentgenogram revealed the crown of an embedded first molar projecting into a cyst. The second and third molars were absent and this area was occupied by a large cystic lesion (Fig. 24, Neg. 78798). The mass from the second molar region consisted of rather dense, moderately cellular stroma, most of the cells being ovoid or stellate. Interspersed were islets of adamantine epithelial cells, arranged in cords and strands (Fig. 25, Neg. 78580,  $\times 105$ ), some resembling tooth buds (Fig. 26, Neg. 78683,  $\times 70$ ). Some of these islets showed early central cystic degeneration. In one area, an attempt at tooth formation was seen; an inner enamel layer and stellate reticulum could be recognized (Fig. 26); adjacent to this area, a calcified, irregularly formed tissue was being laid down. Several masses of dense hyaline material showed early calcification (Fig. 27, Neg. 78684,  $\times 70$ ). A portion of the tumor, in which there were lobules of epithelial cells, resembled adamantoblastoma.



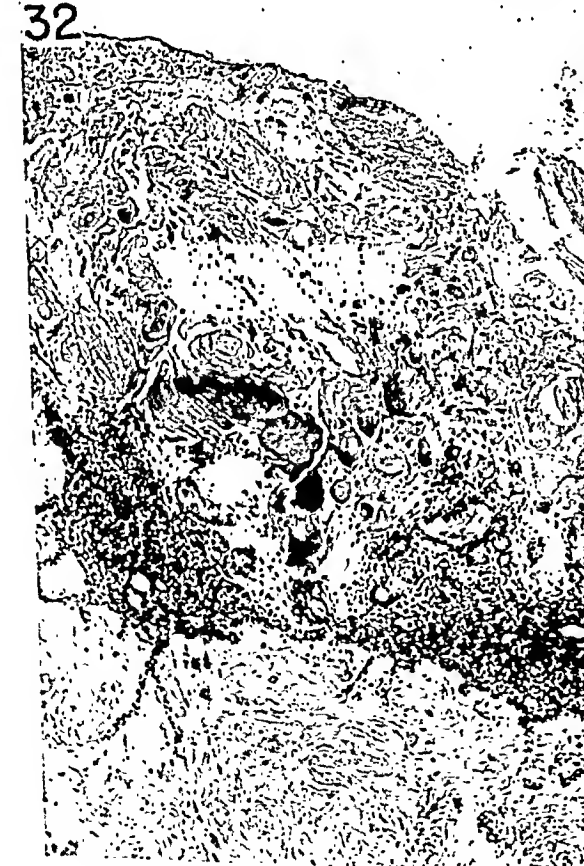
Thoma and Goldman

Odontogenic Tumors



PLATE 96

FIGS. 28 to 33. Case 47. *Odontogenic mixed tumor*. A.I.P. Acc. 133772. A swelling on the right side of the face of a boy, 15 years old, was confined to the ascending ramus. Gradual increase in size and slight pain noticeable for 9 months were attributed to a blow. On the right side of the mandible the second and third molars were missing. The roentgenogram showed a two-compartment cyst: the first, below the roots of the first molar, contained a tooth displaced to the inferior border; the second, which occupied the entire ramus and expanded its anterior border, contained a mass of round calcified particles (Fig. 28). Microscopically, the cyst capsule (Fig. 29, Neg. 85478,  $\times 4$ ) was composed of dense connective tissue lined by adamantine epithelium, some of which was necrobiotic (Fig. 32, Neg. 85491,  $\times 90$ ). The adamantine cells were, for the most part, packed closely and their nuclei stained deeply. Attached to the adamantine tissue and situated in the capsule were many embryonal tooth organs composed of a layer of ameloblasts surrounding a dental papilla (Fig. 31, Neg. 85494,  $\times 90$ ). An attempt to form an odontoblastic layer was noted. Between the tooth organs was a stellate reticular tissue (Fig. 31), and in some of them enamel and dentin were formed (Fig. 29). Attached to the functional ameloblastic layer was neoplastic adamantine tissue (Fig. 31), the cells of which tended to be arranged around small cysts. In several places in the capsule, masses of irregularly formed dentin with sparse and irregular tubules were laid down; cellular inclusions were found in the dentin (Fig. 30, Neg. 85492,  $\times 100$ ). In one area there was an irregular formation of tooth substance as in a complex odontoma (Fig. 33, Neg. 85493,  $\times 60$ ).

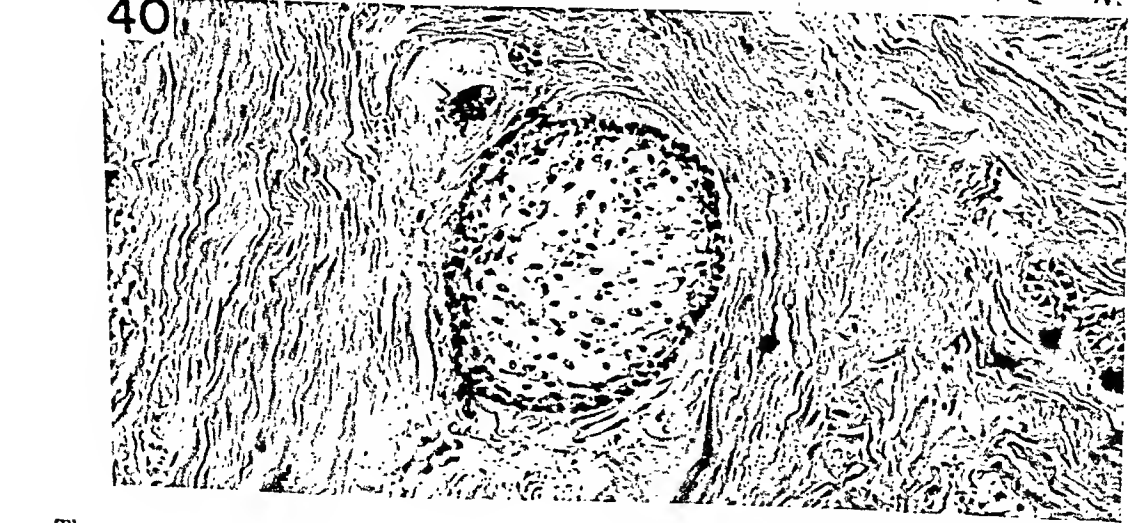
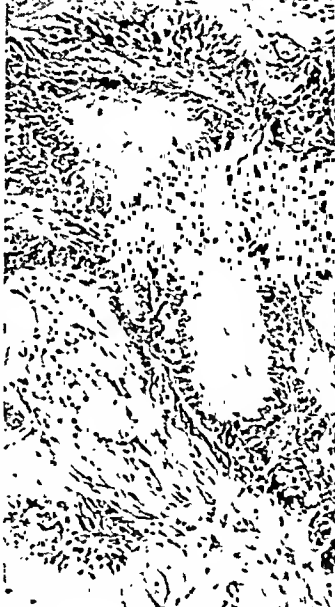
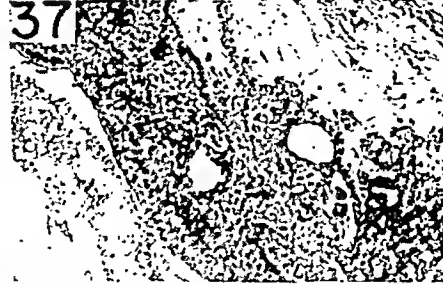
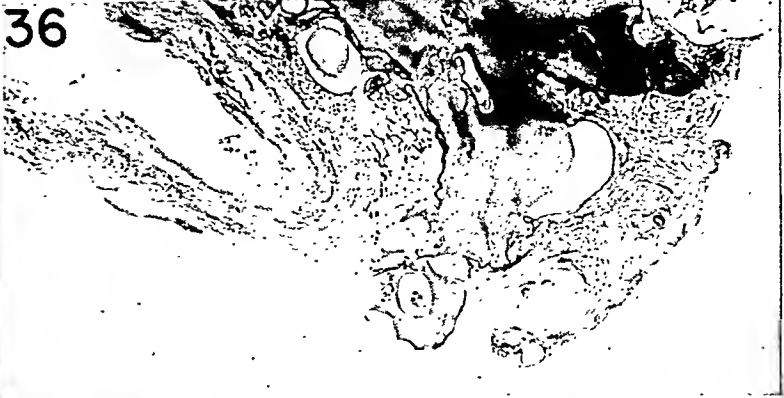


Thoma and Goldman

Odontogenic Tumors

# PLATE 97

FIGS. 34 to 40. Case 46. *Odontogenic mixed tumor*. A.I.P. Acc. 132671. A white woman, 35 years old, had had a painless swelling of the left side of the face for 1½ years (Fig. 34, Neg. 86219). A firm mass over the left ramus of the mandible was shown by roentgenologic examination to contain a large cystic cavity involving the entire left ramus and part of the mandible, and to encase the crown of a molar tooth. Above the tooth were numerous, fused, radiopaque masses (Fig. 35, Neg. 86219). The material removed at operation consisted of an irregular, reddish pink, smooth sheet of membranous tissue and attached fibrous tissue in which several firm, white, smooth, encapsulated nodules were embedded. At one corner of the sheet was an irregular reddish pink, mucosa-covered mass of firm tissue studded with many pearly white, round, smooth calcified nodules. In the center was an abortive tooth. Microscopically, the large calcified mass (described as the abortive tooth root) was made up of irregularly formed dentin and covered by projections of cementum; close by were small rudimentary teeth (Fig. 36, Neg. 85480,  $\times 4$ ). Epithelial membranes surrounded spaces which represented the enamel dissolved during decalcification. Some of the more immature enamel was still attached to a core of dentin which in some instances contained a central pulp canal. Connective tissue surrounded some of the epithelial membrane, and, occasionally, cementum extended from the tooth to join the connective tissue. Lining a cystic space and extending into the connective tissue were masses of small, deeply staining epithelioid cells which had a tendency to form small acini (Fig. 37, Neg. 85501,  $\times 95$ ). At one place, epithelial structures, rimmed by dentinoid, had proliferated into the adjoining tissue (Fig. 39, Neg. 85483,  $\times 100$ ); at another, proliferation of epithelium had produced irregular papillary structures surrounded by connective tissue. The papilla was made up of an outer layer of cylindrical cells and contained a variety of cells, some of which had undergone squamous metaplasia and had formed pseudo-pearls. Rimming many of these epithelial structures was an eosinophilic material which consisted of irregular tubules and a few cellular inclusions in a somewhat fibrillar material. This material was identified as dentin (Fig. 38, Neg. 85505,  $\times 100$ ). Scattered through the connective tissue capsule were lobules of adamantine cells, many groups of which showed central cystic degeneration (Fig. 40, Neg. 86945,  $\times 160$ ).



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FIGS. 41 to 45. Case 50. *Soft and calcified odontogenic mixed tumor*. A.I.P. Acc. 115927. A slight swelling had appeared 3 days before in the premolar region of a man, 26 years of age. Roentgenologic examination revealed an irregular radiopaque area in the premolar region (Fig. 41, Neg. 82117). Some of the tissue removed at operation was hard as bone; some was soft and fibrous. The sections showed a complex arrangement of tooth elements surrounded by areas of *degenerated epithelium* (Fig. 42, Neg. 85497,  $\times 6$ ). The dental structures were irregularly formed, entwined, and sometimes attached by a light-staining, eosinophilic, bone-like substance of the character of osteocementum. The aborted teeth were covered by varying amounts of enamel, the matrix being retained and not entirely removed during decalcification. Cementum covered the coronal portions of the teeth. Attached to the osteocementum and occupying the remaining portions of the specimen were ghost-like epithelial cells (Fig. 43, Neg. 85486,  $\times 315$ ). Situated between the irregular dental structures were masses of epithelial cells, polyhedral or flattened, having a tendency to arrange themselves around small spaces; in some, focal calcification was noted (Fig. 44, Neg. 85487,  $\times 315$ ). In one section which consisted of the capsule of the cyst, the lining was composed of squamous epithelium with spindle-shaped nuclei and epithelium in the acinar arrangement previously described. Islands of adamantine tissue were evident in the fibrous connective tissue capsule and in one area was seen a ring of dentin, its outer aspect covered on one side by adamantine epithelium, its center consisting of long fibers with relatively few cells. Calcification was seen in one area. The dentin contained irregular tubules and an occasional entrapped cell (Fig. 45, Neg. 85504,  $\times 185$ ).



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## PLATE 99

FIGS. 46 to 50. Case 45. *Odontogenic mixed tumor*. A.I.P. Acc. 133773. A boy, 16 years of age, noticed a swelling under his upper lip which had been growing for several months, and tenderness of the left maxillary lateral and central incisors. Bulging of the labial surface of the alveolar process prevented the lips from closing. The swelling was firm but crepitant. The roentgenogram disclosed a cystic area extending from the alveolar process of the anterior part of the maxilla into the palate. In the center was a radiopaque mass, irregular and unevenly calcified (Fig. 46). Microscopically, the cyst was composed of vascular fibrous connective tissue lined by stratified squamous epithelium. Springing from one side of the cyst wall and nearly filling the cavity was a mass of tooth-like material in connective tissue stroma (Fig. 47, Neg. 85497,  $\times 7$ ). Two or three of the structures were nearly normal teeth on a small scale, the others were smaller, less well differentiated, and without pulp centers. Although the dentin was laid down normally, the enamel was not arranged correctly. Necrobiotic epithelial cells arranged in concentric masses, resembling the spider cells of an adamantoblastoma, were found largely between the small, poorly differentiated teeth (Fig. 48, Neg. 87684,  $\times 70$ ). In some of the masses, focal areas of calcification were seen. Aggregations of adamantine epithelial cells were scattered among collections of dentin and epithelial ghost cells. Most of this adamantine tissue was solidly packed but in some areas it assumed a cystic pattern, the spaces being filled with a colloid-like material. Here and there dentin had been laid down haphazardly. In the capsule were accumulations of the same necrobiotic epithelial cells as in the lumen, also lobules of epithelial cells of distinctly adamantine nature. The peripheral cells were either cuboidal or columnar, resembling the prefunctional ameloblast (Fig. 49, Neg. 85503,  $\times 430$ ). Of greatest interest was the formation of a small tooth bud in the cyst capsule. Structurally it was composed of epithelium, dentin, and dental papilla, but the epithelium did not suggest functional ameloblasts and there was no attempt at enamel formation. A considerable layer of dentin was evident, although the odontoblastic layer was not regular or even fully developed; the dental papilla was characteristic (Fig. 50, Neg. 85484,  $\times 85$ ).





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PLATE 100

FIGS. 51 to 56. Case 52. *Odontogenic mixed tumor*. A.I.P. Acc. 127807. A boy, 8 years old, had a diastema between the maxillary central incisors and a hypertrophied labial frenum. On the right were the two permanent incisors, on the left the two retained deciduous ones. Roentgenologic examination disclosed a calcified mass (Fig. 52, Neg. 85191), distal to which were three unerupted teeth. Six years before, the left central deciduous incisor failed to erupt normally and a small cystic lesion was noted in the roentgenograms but was not removed (Fig. 51, Neg. 85191). This history suggested an odontoma which had formed in the cystic area and which probably had at first been soft but later had expanded to form calcified dental structures. Microscopic examination showed that the mass was composed of the various elements of the tooth, arranged in the irregular pattern consistent with odontoma (Fig. 53, Neg. 85556,  $\times 6$ ), and was encapsulated by fragmentary connective tissue which in one area contained adamantine epithelium arranged in acini filled with a mucoid substance. Lying in dentin or enamel spaces were collections of ghost epithelial cells which had undergone calcification (Fig. 54, Neg. 85553,  $\times 180$ ). In some areas there was evidence of necrobiosis of the epithelium preceding calcification. Attached to the normal dentin were masses of irregularly formed osteodentin around ghost-like epithelial cells (Fig. 55, Neg. 85555,  $\times 120$ ). At one place remnants of an ameloblastic layer lay over an enamel space; this layer was adjacent to flattened epithelium which inclined toward an acinar arrangement (Fig. 56, Neg. 85558,  $\times 480$ ).



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FIGS. 57 and 58. Case 59. *Odontogenic mixed tumor*. A.I.P. Acc. 105338. A man, 24 years of age, had been aware of an asymptomatic swelling of the face for about 1 year. There was an edentulous area distal to the first premolar. The roentgenogram revealed a large calcific mass, beneath which there was a molar tooth. An osteolytic area suggested that the mass was lying in a cyst (Fig. 57, Neg. 78297). Microscopically, a diffusely pink substance contained numerous excavations which made it appear cancellous (Fig. 58, Neg. 78793,  $\times 8$ ). For the most part this substance was dentin; adjacent, and lying free in some of the spaces, was cementum. Cementicles were present, especially in areas which tended to simulate periodontal membrane. There was a tendency to form regular tooth structure; however, this differentiation was not achieved. The numerous excavations were enamel spaces. Partially surrounding the mass was a narrow zone of granulation tissue and beyond this, one of dense young connective tissue.

FIGS. 59 and 60. Case 58. *Odontogenic mixed tumor*. A swelling of the right side of the face of a man, 20 years of age, was asymptomatic and of a few years' duration. Roentgenologic examination revealed a large calcified mass, consistent with odontoma, extending from the second molar region back to the ramus, and down almost to the border of the mandible. Lying posterior to this mass was a cystic area into which the crown of an impacted third molar protruded (Fig. 59, Neg. 78795). In sections, spicules of dentin arranged in a heavy network were lined in some areas by cementum. Between the spicules there were oval, elongated, usually empty channels, some of which, however, contained remnants of enamel. There were spaces where the enamel had been dissolved by decalcification. At one edge, an embedded nodule resembled a portion of a distorted tooth with a dentin core, an enamel cap, a pulp chamber with a regular odontoblastic layer, and a few denticles lying in the pulp (Fig. 60, Neg. 78796,  $\times 6$ ). The attached capsule was composed of connective tissue in which strands, lobules, and cords of adamantine epithelial cells were present. In one area a cystic structure was seen.

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Odontogenic Tumors



## ATYPICAL LICHEN PLANUS\*

JULIUS ROSENTHAL, Major, M.C.†

(From the Schick General Hospital, Clinton, Iowa)

### INTRODUCTION

I have had opportunity to examine a number of specimens taken for biopsy from patients who were being treated for a skin disease which had its origin in the Southwest Pacific area and which, for lack of a better designation, has been called atypical lichen planus, or lichenoid dermatitis. Since beginning this study I have also been able to examine one patient who presented similar clinical and histologic findings, but whose disease began in Italy. Clinically, all these cases showed various manifestations: papular, eczematoid, nodular, or hypertrophic. Pathologically, they also presented a variety of features depending on their duration or severity. In all, 21 specimens were examined.

Although the cases cannot be divided into precise categories because the disease has a gradual evolution from acute involvement to final healing, nevertheless, on a morphologic basis, it has been possible to classify the microscopic picture roughly into three stages. These have been called (1) acute, (2) subacute, and (3) chronic or healing.

Histologically, these stages were seen to merge with one another so that occasionally the central part of a section would indicate one stage of the disease while the peripheral portion would have a different appearance. Nevertheless, the classification is of some value, for besides indicating the severity of the process in the sections examined, it also calls attention to the fact that more than one histologic pattern can be expected in a biopsy examination. Of the 21 specimens examined, 6 were considered as essentially acute, 7 as essentially subacute, and 8 as chronic.

To illustrate these three gradations, three fairly typical examples have been chosen.

### ILLUSTRATIVE CASES

#### Case 1 (Acute Stage)

This patient was a staff sergeant in a baking company. He left the United States on September 21, 1943, and arrived in Brisbane, Australia, on October 23, 1943. He remained there 6 weeks and then left for New Guinea. He arrived in Milne Bay on December 1, 1943. He remained there for 2 months and then went to Oro Bay, where he arrived on February 10, 1944.

*Diet.* While he was stationed in Australia, he had a very adequate diet, including fresh meat, vegetables, and fruits each day. His diet in New Guinea, he stated,

\* Received for publication, October 27, 1945.

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seemed quite adequate. He had fresh meat four or five times a week and fresh vegetables and fruit quite often.

*Atabrine.* Atabrine was started on November 20, 1943. The dose was 0.1 gm. daily until December 1, 1943, when it was increased to 0.2 gm. daily. About three times a month he would take six tablets a day for 3 days. These were periods after he had been in the jungle and had been bitten by mosquitoes. He had no symptoms to suggest atabrine sensitivity.

*Tropical Diseases.* He had no malaria, dengue, scrub typhus, or tropical ulcer. On one occasion he was bitten by many small red ants but knows of no other insect bites.

*Geographical Location.* He was stationed at Milne Bay and Oro Bay. His campsite at Oro Bay was located in an area that had already been cleared. It was near swampy land where there were many trees. He went into the jungle on several occasions to cut and peel trees. During these periods he was exposed to vegetation to a rather marked degree. He stated that natives came to work in their camp area quite frequently, but at no time did he get any closer to them than 3 feet.

His skin trouble started on March 1, 1944, when, following peeling of small trees and getting some of the sap on his wrists, he noticed itching at those regions. About 10 days later he developed a fine vesicular eruption over the dorsum of both hands. He entered the hospital on April 22, 1944, with a mild fever and a rather marked edema over the dorsal surface of both hands. These regions were quite tender. He was transferred to a general hospital where examination on April 27, 1944, revealed a generalized, lichenoid, papular eruption with bullous lesions on the lips and buccal mucous membrane. He was evacuated to the zone of the interior with his skin condition improved, but he noticed an increase of pigmentation.

*Physical Examination.* Examination revealed an undernourished soldier who appeared older than the stated age. There was a heavy atabrine pigmentation of the skin. There was a markedly erythematous, generalized, papular and nodular eruption which was accentuated on the extensor surfaces of the elbows and knees, as well as over the sacrum and the heels. Numerous lesions of the same type were present on the scalp, especially on the posterior portion where there were areas of patchy hair loss. There were many small, milky white papules on the mucous membranes of the cheeks. The rest of the general physical examination was negative.

### Microscopic Description

Sections (Fig. 1) showed a central area with marked cellular infiltration and peripheral regions of more chronic inflammation.

The stratum corneum was variously thickened. In some areas this thickening was prominent; in others it appeared only as a few strands of horny tissue. The openings of the hair follicles were occasionally seen to be dilated and to be filled with masses of keratin, some of which contained the remnants of nuclear material. No definite parakeratosis was seen. The granular layer also appeared variously involved. In some areas it was almost completely absent; in others it consisted of several layers of granular cells ranging in number to seven. One region of the section showed a marked acanthosis with marked keratin production and the formation of horny plugs. In this region, the basal layer was hypertrophied, contained little pigment, and was surrounded by masses of inflammatory cells. The cells in

the acanthotic areas, and more particularly in the lower layers of the rete, showed intracellular and intercellular edema with vacuolization and widening of the intercellular spaces. In this region, the lower portions of the rete appeared infiltrated with inflammatory cells, indistinct, and blended into the inflammatory exudate. Over the remainder of the section where the inflammatory exudate was not marked, the rete appeared narrow. Its pegs were shortened and their margins indistinct from the surrounding tissue.

The papillary layer of the corium in the granulomatous areas was infiltrated with masses of cells which rarely invaded the epidermis for a short distance. The cellular collections consisted principally of histiocytes and lymphocytes, the majority seemingly being histiocytes. Polymorphonuclear cells were present, chiefly as eosinophils. Polymorphonuclear neutrophils were very rare. Collections of pigment were found in the lower papillary layer, particularly in areas where the cellular accumulation was not marked. However, collections of pigment were also found in the tips of the papillae where the infiltration was considerable. The connective tissue of the papillary and subpapillary layers appeared thickened in the noncellular areas and replaced by cells in the inflammatory ones. The lower layers of the cutis as well as the subcutaneous tissue showed few abnormalities. The connective tissue was not increased in amount. Small collections of chronic inflammatory cells were occasionally seen around hair follicles or in the vicinity of sweat glands. No marked vascular changes were seen.

#### Comment

The acute stage is marked principally by an acute inflammatory exudate, limited to the papillary and subpapillary connective tissue, which extends along the hair shafts, and consists of large numbers of lymphocytes and histiocytes. There is marked acanthosis and hyperkeratinization with plugging of the openings of the hair follicles. The stratum basalis is edematous, liquefied, and infiltrated with large numbers of inflammatory cells, mostly lymphocytes and histiocytes. Large collections of pigment are frequently found at the tips of the papillae. Such collections may also be found in the subpapillary layers. The subcutaneous tissue is not involved.

#### *Case 2 (Subacute Stage)*

This soldier was in the military police and was assigned to the Fifth Air Corps. He left the United States on May 25, 1943, and arrived in Brisbane, Australia, on June 13, 1943. He left Brisbane for New Guinea on August 31, 1943, and arrived in Port Moresby on September 10, 1943. He remained there until October 27, 1943, and at that time flew to Gusap, New Guinea, arriving there the same day.



*Diet.* While in Australia, he received a fully adequate diet, consisting of fresh meat, vegetables, and fruits almost every day. After arriving in New Guinea, he had a very satisfactory diet for the first 3 months. He had fresh meat almost every day and sometimes twice a day. He had apples and oranges almost every day. Subsequently, he had mainly C rations, although about once or twice a week he had fresh meat, and during this period he received practically no fresh fruits or vegetables.

*Atabrine.* Atabrine was started on August 31, 1943. Dose was 0.1 gm. daily, for the most part. For short periods of time he would take 0.2 gm. daily. Atabrine was discontinued on May 16, 1944, except when it was given therapeutically for malaria. He had had five attacks of malaria, the last being in June, 1944, and with each attack he had received atabrine. Neither malaria nor atabrine medication seemed to have any influence on the clinical course of the lichen planus. He had no symptoms to suggest sensitivity to atabrine.

*Tropical Diseases.* The patient had had malaria as described but no dengue, scrub typhus, nor tropical ulcer.

*Geographical Location.* At Gusap, New Guinea, his camp was located in the woods for the first month. He then moved out into a flat area that was covered with kunai grass. He went into swampy areas on various occasions and helped clear the jungle when the original camp was set up. He handled a moderate amount of vegetation during this period. Natives worked around their camp area and he went into native villages on a few occasions. He had worked side by side with natives but at no time did he touch them.

His present illness began after an attack of malaria in December, 1943. One week later his feet and legs began to itch. During this period he noticed that his skin seemed to bruise easily and that local infections seem to be present for long periods of time. He also noticed the development of three white sores on his lips, which had indefinite borders. On March 12, 1944, he developed swelling of his eyelids. He then developed scaling lesions over his lower legs which spread to involve most of the body. Soon he developed patchy areas which were elevated, scaled readily, and were well defined. These were grayish white. He was hospitalized on March 13, 1944, and was treated with baths and various local applications without benefit. The eruption continued to get worse. It was decided to evacuate him to the United States and he left New Guinea on May 15, 1944. Since leaving New Guinea, he estimated that he had improved 40 per cent.

*Physical Examination.* Examination revealed a generalized, diffuse, purple-red pigmentation over the skin of most of the body. The skin over his arms and legs was somewhat granular. There were three clean, shallow ulcers on the shaft of the penis. There was a patchy alopecia. There was no evidence of involvement of the mucosal surfaces.

### Microscopic Description

The keratin layer was widened and fragmented (Fig. 2). The increase of keratin was particularly noticeable in the openings of hair follicles. The granular layer was slightly hypertrophied, particularly at the openings of the follicles. The epidermis generally was atrophic. This was particularly noticeable in the rete columns, which were frequently found to be sharpened. The basal layer was at times found to be disorganized and partly liquefied, while at other times it was somewhat hypertrophic. The papillae of the corium contained increased cells, mostly histiocytes, and scattered amounts of pigment, little of which was seen in the basal layer. The cellular infiltrate ex-

tended into the lower portions of the rete, some of the pegs of which were infiltrated and destroyed. Numerous vacuolated spaces were seen in the papillae as well as in the subpapillary connective tissue. Collections of lymphocytes were occasionally seen in the latter, and at times these showed a tendency to perivascular grouping. Slightly increased lymphocytes were seen around a few hair follicles in the lower corium.

### Comment

The inflammatory reaction is still apparent in the subacute stage, but to a much lesser degree. Inflammatory cells in moderate numbers are still present. The outstanding lesion is the degenerative change in the stratum basalis with resultant atrophy and sharpening of the rete pegs. Considerable edema is present both in the lower epidermis and in the corium. Collections of pigment are frequently seen in the papillary or subpapillary connective tissue.

### *Case 3 (Chronic or Healing Stage)*

This soldier was a private in the infantry and sailed for Hawaii in September, 1943. He went to the Southwest Pacific on February 2, 1944. He received atabrine during the time he was there. Two months after his arrival he developed a pruritic, vesicular eruption on the dorsal surface of the mid-finger of the right hand. Three days later an eruption of the same type began on the other hand and the lower legs. This gradually became generalized and more exudative. He entered the hospital on May 9, 1944, and was treated with starch baths and various local treatments. His disease was characterized by periods of improvement followed by periods of exacerbation. He was evacuated to the United States and arrived at Letterman General Hospital on July 29, 1944, and was then transferred to Schick General Hospital with a diagnosis of: lichen planus, hypertrophic, chronic, generalized, severe, cause undetermined.

*Physical Examination.* On examination the skin over the neck, extensor surface of the upper arms, entire forearms, and legs presented a diffuse, very heavily infiltrated, indefinitely outlined, dark brown eruption with much patchy adherent scarring. There were numerous patches of varying size and shape of the same type scattered over the body and in the groins. The mucous membranes were normal.

### Microscopic Description

Marked hyperkeratosis without parakeratosis was present (Fig. 3). Plugging of the hair follicles by keratin was frequently seen. The epidermis varied in thickness. In some areas it appeared atrophic, while in others it was somewhat acanthotic. Variations in size of the rete pegs were seen, some appearing sharpened, while others appeared somewhat acanthotic. The basal layer at times was found to contain an increased amount of pigment. The papillary and subpapillary connective tissue appeared thickened and more homogeneous than the remaining tissue of the corium. Focal collections of inflammatory cells were seen in the subpapillary connective tissue and especially about hair follicles. A large amount of pigment was present in some of the

papillae. Collections of inflammatory cells were seen occasionally around sweat glands and around the hair follicles in the upper regions of the corium.

### Comment

The inflammatory reaction has largely subsided in the chronic stage. Sufficient residua are present, however, to indicate the origin of the lesion. Scattered inflammatory cells as well as occasional small foci are still present in the upper layers of the corium. Some restitution has occurred in the basal layer and some of the rete pegs are of usual width and appearance. Others, however, show persistent degenerative changes in the basal layer with consequent sharpening of their outlines. Small masses of pigment are occasionally seen in the papillary and subpapillary connective tissue. When seen in this stage, it is extremely difficult to differentiate this condition from other toxic dermatoses in tissue sections.

### SUMMARY OF THE THREE CASES

From the above descriptions it can be seen that there are certain microscopic features which are common to the three stages. These are the hyperkeratinization and plugging of the openings of the hair follicles, the degenerative and liquefactive changes in the basal layer, and the presence of cells (their number depending on the severity of the disease) in the papillary and subpapillary connective tissue, around the hair shafts, hair follicles, and around some of the dermal glands. The changes in the basal layer are apt to be patchy, varying in severity in different regions. In the acute stage they are best discernible but even at this stage may not be distinguishable from those of other dermatoses. The cellular infiltrate consists almost entirely of lymphocytes and histiocytes, polymorphonuclear and plasma cells being uncommon. Eosinophils are occasionally seen. Accumulations of pigment are very frequently seen, as will be described below. There are no marked vascular or collagenous changes other than those that accompany acute inflammation. Edema is frequently seen in the acute and subacute stages, and is found both extracellularly and intracellularly in the prickle cell and basal layers as well as in the upper layers of the corium. Atrophy of sebaceous glands and infiltration around sweat glands are frequent.

Of all the changes noted, the one that is common to all stages is the lesion in the basal layer. This is an early as well as a late finding, and this layer of the epidermis is apparently the last to return to normality when the disease has subsided.

*Pigment.* An outstanding feature is the aggregation of pigment in the papillary and subpapillary connective tissue. These collections are

most often seen in the acute cases. Of six acute cases, 5 showed this phenomenon to a marked degree. The pigment is sometimes seen as aggregates of dark masses, most characteristically at the tips of the papillae, but frequently also in the subpapillary connective tissue. The pigment is found mostly in macrophages, although it is also seen scattered in the connective tissue. This pigment gives a negative reaction with stains for hemosiderin or hemofuscin, and gives a positive reaction with the Becker stain for melanin.\* This pigmentation is not limited to the acute cases and even in some of the chronic cases it occurs in appreciable amounts in the papillary or subpapillary connective tissue. The normal pigment of the basal layer is generally found to be increased.

### VISCERAL MANIFESTATIONS

Early, my attention was called to the fact that, in addition to the skin lesions, some patients had general somatic disturbances. These were particularly hematologic, pulmonary, hepatic, and possibly cerebral. They varied in different patients. In the majority no such symptoms were apparent while in others they were the outstanding features of the disease. There are insufficient pathologic data concerning the pulmonary, hepatic, or cerebral findings. A description of the clinical findings will appear in a report by Lt. Col. D. J. Wilson, Chief of the Dermatology Section. Some observations have, however, been made upon the hemal and hematopoietic alterations.

### HEMATOLOGIC FINDINGS

#### ANALYSIS OF FINDINGS

There were available 18 cases which had various blood examinations. These are summarized in Tables I to III. Changes in the cellular elements as well as in the chemical constituents of the blood were encountered. A moderate anemia was found in 12 of 18 cases examined (Table I).

#### \* Becker's Stain for Melanin

(*Arch. Dermat. & Syph.*, 1927, 16, 259-290)

1. Run down through water in the usual way.
2. Wash in doubly distilled water.
3. Stain in 2% silver nitrate for 2 hours (2 gm.  $\text{AgNO}_3$ , 100 cc. doubly distilled water) in dark oven,  $37^\circ \text{C}$ .
4. Wash quickly in doubly distilled water.
5. Treat with aqueous solution of sodium thiosulfite, 1 min. (6 gm. to 300 cc.  $\text{H}_2\text{O}$ ).
6. Counterstain in Harris' hematoxylin, 2 min.
7. Dip in acid alcohol.
8. Wash.
9. Saturated solution of lithium carbonate (until tissue is a bright blue).
10. Wash in tap water and 95% alcohol.
11. Dehydrate in acetone.
12. Carbol xylol, xylol, mount in balsam.

TABLE I  
Blood Counts in Cases Studied

No.	Date	Hemo- globin	Red blood cells	White blood cells	Differential count						
					Poly.	Lymph.	Mono.	Eosin.	Late meta.	Baso.	Others
1.	8/9/44	93%	4,510,000	5,700	69	16	3	12			
2.	10/12/44	86%	4,220,000	4,950	57	43					
3.	9/30/44	103%	5,010,000	29,450	67	11	5	17			
	10/17/44	69%	3,130,000	14,450	47	21		32			
	10/24/44	72%	3,530,000	8,150	55	26	3	8	5	1	2 Myel.
4.	NA										
5.	10/14/44	86%	4,450,000	6,600	49	46		5			
6.	10/12/44	93%	4,680,000	8,250	76	14	7	3			
	12/7/44			6,850	65	28		5		1	
7.	10/1/44	97%	4,840,000	8,400	60	33	1	5		1	
8.	NA										
9.	10/11/44	90%	4,110,000	4,650	37	45	5	13			
	9/6/44			6,500	59	31		9		1	
10.	11/3/44	86%	4,420,000	7,300	57	31	3	7		2	
11.	10/30/44			8,900							
12.	12/7/44	100%	4,890,000	7,900	46	50		4		1	
	1/6/45	90%	4,030,000	7,900	68	25		6		1	
13.	12/18/44	72%	3,430,000	4,700	57	34	1	8			
	11/22/44			3,500	45	43		12			
14.	12/6/44	100%	4,960,000	5,200	43	51		6			
15.	12/20/44	100%	4,670,000	5,400	74	20		6			
	12/5/44			7,400	79	16		5			
16.	11/22/44	97%	4,880,000	6,100	49	46	2	2		1	
17.	9/4/44	70%		7,700	64	18	7	11			
18.	9/28/44	83%		5,900	65	30		5		1	
19.	10/30/44	20%	980,000	3,400	36	63		1			
	11/11/45	76%	3,250,000	4,100	33	66	1				
20.	2/5/45	93%	4,410,000	5,100	66	28	2	3		1	
21.	11/14/44	90%	4,540,000	2,700		94		5			1 Myel.
	11/20/44	58%	3,050,000	1,540	3	91		5			

NA=Records not available.

Of interest is the number of cases having lymphocytosis and eosinophilia in one or more counts. Twelve of the 18 cases, or 66 per cent, showed lymphocytes of 30 per cent or over, while 13 of the 18 cases, or 72 per cent, showed an eosinophilia of 5 per cent or over. The term

TABLE II

*Determinations of Certain Chemical Constituents of the Blood*

No.	Date	Nonprotein nitrogen (mg./100 cc.)	Total protein (per cent)	Albumin (per cent)	Globulin (per cent)	Albumin- globulin ratio
1.	10/14/44		6.4	5.2	1.2	4.3
2.	9/27/44	40.4				
3.	10/21/44 10/17/44	31.36	5.34	3.18	2.16	1.47
4.	NA					
5.	10/14/44		6.2	3.95	2.25	1.75
6.	10/12/44		8.7	5.2	3.5	1.5
7.	10/1/44		8.6	5.2	3.4	1.53
8.	NA					
9.	10/11/44 10/17/44	42.46	7.8	5.2	2.6	2.0
10.	NA					
11.	NA					
12.	NA					
13.	11/22/44	27.0	5.15	2.76	2.39	1.15
14.	NA					
15.	NA					
16.	NA					
17.	NA					
18.	NA					
19.	12/5/44		6.92	4.46	2.46	1.81
20.	NA					
21.	12/10/44	43.0				

NA=Records not available.

lymphocytosis is used to designate a count of 30 per cent or over. By the term eosinophilia is meant an eosinophile count of 5 per cent or more.

Changes in the chemical constituents of the blood were encountered occasionally. These consisted particularly in a decrease in the total protein and in a lowering of the albumin-globulin ratio (Table II).

It must be mentioned that these figures cannot be considered as representative since only the more severe cases and particularly those

with some complication had complete blood studies. In a complete statistical study of unselected cases the figures would undoubtedly be much lower.

#### APLASTIC ANEMIA

Two cases had severe hematopoietic disturbance; one of these has improved markedly, while the other terminated fatally.

#### Case 4

The patient was a white male, 23 years old, who was admitted to Schick General Hospital on October 2, 1944, with a generalized skin eruption. Previous medical history was irrelevant. He had entered the Army on October 15, 1942, and was transferred to New Guinea in January, 1944. Two months after his arrival he developed a dry, scaly eruption on the dorsum of the right hand which eventually spread and became generalized. This condition had periods of remission and exacerbation. He was finally evacuated to the zone of the interior and arrived at Schick General Hospital on October 2, 1944, with a transfer diagnosis of dermatitis, chronic, eczematoid, generalized, severe.

Examination on admission at Schick General Hospital showed some evidence of weight loss and marked atabrine discoloration of the body. There was a patchy, fairly well outlined, erythematous, moderately infiltrative, scaly dermatitis with many small punched-out ulcerations, some bleeding, on the lower third of both legs and dorsal aspects of feet. Hemoglobin on admission was 93 per cent, and white blood cell count, 5,250. The diagnosis of lichen planus, New Guinea, was made.

On October 12, 1944, he developed a chill and on October 13, 1944, malarial parasites (*vivax*) were found in the blood. He was treated with atabrine, and completed his malarial therapy on October 20, 1944, with disappearance of parasites from the blood and remission of his symptoms. On October 30, 1944, he complained of weakness and tiredness. He appeared anemic. An examination of the blood was reported as follows: hemoglobin, 20 per cent; red blood cells, 1,280,000; white blood cells, 2,400; color index, 0.82; platelets, 70,000; differential: neutrophils, 36 per cent; lymphocytes, 63 per cent; eosinophils, 1 per cent. He was treated with transfusions, liver, and iron with considerable improvement.

Bone marrow cell count, done on January 17, 1945, was as follows: metamyelocytes, early, 1.5 per cent; metamyelocytes, late, 3.5 per cent; polymorphonuclear neutrophils, 18 per cent; polymorphonuclear eosinophils, 0.5 per cent; small lymphocytes, 65 per cent; large lymphocytes, 11.5 per cent.

The patient improved rapidly and on February 1, 1945, blood count was as follows: red blood cells, 4,650,000; white blood cells, 5,550; hemoglobin, 97 per cent; differential: neutrophils, 24 per cent; lymphocytes, 73 per cent; monocytes, 3 per cent; slight anisocytosis and poikilocytosis; reticulocytes, 1.7; platelets, 130,000. Other laboratory studies were as follows: Kahn test, negative; hematocrit, 46 per cent; volume index, 1.07; icteric index, 4.9; cephalin flocculation test, negative; hippuric acid excretion test, 3.51 gm. (normal, 2.55 to 3.3); serum protein, 6.92; serum albumin, 4.46; serum globulin, 2.46 per cent.

In summary, this was a case of lichen planus which developed symptoms and blood findings of aplastic anemia shortly after an attack of malaria for which he received an intensive course of atabrine therapy. While his blood picture had not become entirely normal, it had improved sufficiently to warrant the belief that it would become so.

*Case 5*

The patient was a white male, 33 years old, a captain, who was admitted to Schick General Hospital on November 11, 1944. Family and previous history were irrelevant. He had entered the Army on January 19, 1943.

*Present Illness.* On about April 1, 1944, approximately 7½ months before admission to Schick General Hospital, while in New Guinea, he had developed a generalized skin eruption which was diagnosed as lichen planus. He was treated with various external applications, with improvement. He continued to take atabrine up to October 1, 1944. In October, 1944, he noted gradual onset of fatigue, weakness, headache, numbness and tingling sensations of the extremities. He was admitted to the Station Hospital in the Southwest Pacific where on October 21, 1944, blood count showed: red blood cells, 1,000,000; white blood cells, 2,700; polymorphonuclear cells, 22 per cent; lymphocytes, 78 per cent; hemoglobin, 60 per cent; platelets, markedly diminished. A diagnosis of aplastic anemia was made. He was evacuated to the zone of the interior and was admitted to Schick General Hospital on November 11, 1944. Here the lichen planus was found to be largely healed. The diagnosis of normochromic anemia of the aplastic type was corroborated.

He showed no response to vigorous therapy, including pentnucleotide and repeated transfusions, developed multiple hemorrhagic phenomena, and died as a result of cerebral hemorrhage.

*Post-Mortem Findings*

The body was that of an adult white male of good development and nutrition. There were seen ecchymoses of both palpebrae and especially of the left, with marked swelling, a thin bloody discharge from the nose, small ulcers of gums, and small ecchymoses of the buccal mucosa. There were no palpable lymph nodes. Sclerae showed a slight icteric tint. There were numerous ecchymotic hemorrhages of the face, trunk, shoulders, and extremities which ranged to 2.0 cm. in diameter. No palpable abdominal masses or viscera were found. The anterior muscles of the trunk were dark red, well developed, and contained numerous large, ecchymotic hemorrhages which ranged to 3.0 cm. in diameter.

*Heart.* The visceral pericardium showed scattered small ecchymoses. The epicardium contained numerous black ecchymoses. The valves were natural. The auricular and ventricular cavities were of usual appearance. The myocardium of the left ventricle was pale brown and on section showed occasional, isolated petechial hemorrhages. The coronary vessels were patent throughout and showed slight superficial atheromatous patches. The aorta showed scattered, slight, superficial lipid deposits.

*Spleen.* The spleen was enlarged, weighing 225 gm. The surface was smooth; the capsule not thickened. The organ was soft to palpation. On section, it was seen to be congested.



*Liver.* The liver weighed 2500 gm. The capsule was not thickened, and the surface was smooth. On section, the organ had a pale gray-brown appearance. No areas of necrosis were seen. The liver was not congested. A few subcapsular ecchymoses were present.

*Gallbladder.* The gallbladder measured 5.5 by 2.2 cm. It contained yellow bile and no stones. The extrahepatic ducts were patent.

*Stomach.* The stomach was markedly dilated and contained 250 cc. of undigested food. The mucosa was studded with numerous hemorrhages varying in size from 0.1 to 0.5 cm. in diameter. There were seen also numerous brownish marks on the serosal surface, apparently from previous hemorrhages.

*Kidneys.* Weight of the left kidney was 225 gm. It was surrounded by an abundant perirenal fat, in which there was a hemorrhagic area 3.5 by 3.5 cm. The capsule stripped with ease, leaving a smooth surface. The architecture of the kidney was well maintained. The cortex over the pyramids was 0.8 cm. in thickness. The width of the pyramids was 2.0 cm. In the pelvis and upper calyces there was found a recent hemorrhage which extended to the upper portion of the ureter. The right kidney weighed 250 gm. and resembled the left in all respects. The pelvis and calyces were filled with a large hemorrhage which also extended into the upper portion of the ureter.

*Adrenals.* The right adrenal weighed 12 gm. The cut surface showed marked autolysis of the medulla. The cortex was narrow and yellow. Surrounding both adrenals in the retroperitoneal fat were large ecchymoses. The left adrenal weighed 7 gm. It was similar to the right.

*Sternal Bone Marrow.* The usual amount of marrow was obtainable. It was pale brown, somewhat more fluid than usual and contained a considerable amount of fat.

*Lungs, Pancreas, Thyroid, Pituitary Body, Small and Large Intestine, Bladder, Prostate* showed no significant gross alterations.

*Brain.* Weight of the brain was 1721 gm. The vessels on the surface of the brain were markedly congested. An area of ecchymosis, 2.0 by 2.0 cm., was seen lying within the angular gyrus on the left side. At the base of the brain a large subarachnoid hemorrhage was found covering the pons, midbrain, medulla, and the posterior portion of the cerebellar hemispheres. On section, all ventricles were found to be filled with blood. The basal hemorrhage was seen to extend into the interpeduncular space. The lateral ventricles were distended with massive hemorrhage and the septum pellucidum was ruptured. The vessels of the circle of Willis were all embedded in the hemorrhage on the surface of the brain. No definite abnormalities were seen in them.

## Microscopic Description

*Skin.* Sections of skin were cut along the line of the "Y" incision of the chest and abdomen. Small areas of hyperkeratosis were present, extending into the openings of the hair follicles. The granular layer was not well visualized. The stratum spinosum consisted of a few layers of cells. The basal layer varied in appearance. In some regions it was well outlined, in others the demarcation between the basal layer and corium was indistinct. The tips of the rete pegs were found in some regions to be partially liquefied. Some of the individual cells showed intracellular edema. A number of scattered histiocytes were present in some of the papillae and in areas of the subpapillary connective tissue. Scattered collections of pigment were occasionally seen in the papillary and subpapillary tissue, mostly in the latter. Such small masses were mostly in chromatophores, although a few granules were seen in the connective tissue itself. Small collections of lymphocytes and histiocytes were seen also in the vicinity of a few sebaceous glands and occasionally near a few sweat glands.

*Myocardium.* The myocardial muscle fibers were generally intact. A slight increase of connective tissue was occasionally seen about some of the smaller blood vessels. In one area there was evidence of a chronic focal myocarditis around an area of necrosis. This was composed of necrotic muscle fibers in which were found masses of bluish granular material. The smaller blood vessels showed no marked changes. Another section of myocardium including mitral valve showed no marked abnormalities.

*Spleen.* The splenic capsule was not thickened. The fibrous trabeculae were of usual width. The follicles were decreased in number and in size. The pulp was of usual appearance, containing numerous macrophages, a few of which were multinucleated. There was a marked increase of brown pigment (hematoxylin and eosin stain) found mostly in the macrophages. The fibrous structure of the organ was not increased in amount. The blood vessels were of usual structure. Iron stain of the spleen showed it to contain a large amount of iron in the pulp.

*Liver.* The liver capsule was not markedly thickened. Underneath the capsule, however, there were seen small areas of degeneration, fibrous tissue replacement, and chronic inflammatory cells. The last consisted principally of lymphocytes and fibroblasts. The general architecture of the liver was preserved, but changes were found in the parenchyma. These consisted of: (Fig. 4) (1) areas of separation of liver cords due to edema; (2) periportal degeneration of liver tissue and concomitant infiltration by numerous lymphocytes and some fibro-

blasts; (3) slight proliferation of bile ducts in the infiltrated areas; (4) localized collections of masses of pigment, gathered mostly in macrophages, in periportal regions. This pigment had a yellowish brown appearance (hematoxylin and eosin stain). It was found mostly in macrophages which were aggregated in foci. The pigment was granular but the individual particles varied in size. While it resembled bile pigment in some areas, it nevertheless was not seen in the bile canaliculi. Its presence in macrophages was prominently noticeable. Iron stain showed it to be negative for iron. With Becker's stain it gave the same reaction as melanin. (However, Becker's stain is not specific for melanin and apparently will also stain bile pigment.) This pigment was also found in the Kupffer cells which were very prominent throughout the liver substance. The latter also contained a considerable amount of iron-positive material.

*Kidney.* The renal capsule was not thickened. Many of the glomerular spaces were dilated, some containing fluid. The tubules were somewhat dilated, particularly the proximal convoluted ones. Some contained a small amount of frothy material. The interstitial tissue was not increased. The blood vessels showed no abnormalities. There was a large recent hemorrhage in the pelvic connective tissue and fat.

*Thoracic Lymph Node.* A thoracic lymph node contained a large amount of anthracotic material. No other pigment was seen with the hematoxylin and eosin stain. The general structure of the lymph node was maintained but the follicles were not well outlined. The blood sinuses were dilated and were occasionally seen to contain large histiocytic cells, some bearing pigment. Iron stain showed the lymph node to contain a considerable amount of hemosiderin.

*Vertebral Bone Marrow.* The bony trabeculae of the vertebral marrow were of normal appearance. There was a marked decrease in the number of cells and a corresponding increase of adipose tissue so that the marrow appeared as a mass of fat in which marrow cells were found isolated or in small groups. These were of two principal varieties: (1) small cells resembling lymphocytes; (2) larger cells containing a considerable amount of homogeneous reddish cytoplasm (hematoxylin and eosin stain) which surrounded darkly stained homogeneous nuclei, which were sometimes found to be eccentric. Multinucleated cells were seen only very rarely. A considerable amount of pigment was present. Iron stain showed a marked increase of iron pigment throughout the section. The appearance of the marrow was that of a marked hypoplasia with some erythroblastic regeneration. The bone marrow cell count (200 cells counted) gave: megaloblasts,

0.5 per cent; early erythroblasts, 3.0 per cent; degenerated early erythroblasts, 1.5 per cent; polymorphonuclear neutrophils, 0.5 per cent; lymphocytes, 92.5 per cent; monocytes, 2.0 per cent (28.5 per cent degenerated cells per 100 white blood cells counted).

The *sternal marrow* was similar in appearance to the vertebral marrow.

*Brain.* Section of various parts of the brain showed no marked parenchymatous changes. The hemorrhages noted on gross examination were limited to the subarachnoid regions and to the ventricles.

*Blood Culture (Post-Mortem).* A hemolytic *Staphylococcus aureus* and hemolytic streptococcus were isolated.

*Final Diagnoses.* (1) Lichen planus (New Guinea); (2) aplastic anemia; (3) focal myocardial hemorrhages and necrosis; (4) bilateral pleural effusion; (5) pulmonary edema; (6) congestion of spleen; (7) hepatitis, subacute; (8) bilateral pelvic hemorrhages in kidneys; (9) subarachnoid and intraventricular hemorrhages; (10) hypoplasia and fatty change of bone marrow; (11) multiple ecchymoses of skin, voluntary muscles, diaphragm, and viscera.

#### Comment

This patient's disease began with a skin lesion which was diagnosed as lichen planus (New Guinea) about 6 months prior to his terminal illness. On about October 1, 1944, he was admitted to a station hospital in the Southwest Pacific where a diagnosis of aplastic anemia was made. This patient continued to take atabrine for the 6-month period between the onset of lichen planus and the development of anemia. On admission to Schick General Hospital on November 11, 1944, the lichen planus was found to be largely healed. The anemia had improved but he still had agranulocytosis and decreased platelet count. He showed no response to vigorous therapy, developed multiple hemorrhagic phenomena, and died as a result of a large cerebral hemorrhage.

At autopsy numerous hemorrhages were found including ecchymoses of skin, kidney, and brain. No nodular lesions were seen in the skin, but skin along the line of incision showed changes interpreted as residua of the previous disease. The marrow showed hypoplasia with predominant decrease of granulocytic elements.

Of special interest in the case was the liver lesion. There was subacute hepatitis involving chiefly the peripheral portions of the lobules, characterized by degeneration of liver tissue and accumulation of chronic inflammatory cells. In addition, isolated masses of pigment were found which were iron-negative and melanin-positive (Becker stain). The Kupffer cells also contained large amounts of pigment.

It is assumed that this pigment is bile pigment. However, there is some resemblance to the melanin pigment of the skin. With ultraviolet light the liver showed a marked greenish fluorescence. The presence of this marked fluorescence in a pathologic liver of a patient who had not had atabrine for 6 weeks prior to his death again called attention to the possibility of atabrine being a factor in this disease. Since, however, this is the only case that I have seen, no definite conclusions are warranted.

TABLE III  
*Analysis of Cases Examined*  
(*Type of Lesion, History of Malaria, Presence of Anemia,*  
*Lymphocytosis and Eosinophilia*)

No.	Type of inflammation in section	Malaria	Atabrine taken	Anemia	Lymphocytosis	Eosinophilia
1.	Acute	NA	+	-	-	+
2.	Acute	-	+	+	+	-
3.	Acute	-	+	+	-	+
4.	Chronic	NA	+	NA	NA	NA
5.	Subacute	+	+	+	+	+
6.	Subacute	+	+	-	-	+
7.	Chronic	+	+	-	+	+
8.	Acute	+	+	NA	NA	NA
9.	Subacute	-	+	+	+	+
10.	Chronic	NA	+	+	+	+
11.	Subacute	-	+	NA	NA	NA
12.	Subacute	+	+	+	+	+
13.	Acute	+	+	+	+	+
14.	Chronic	+	+	-	+	+
15.	Chronic	-	+	-	-	+
16.	Chronic	-	+	-	+	-
17.	Acute	+	+	+	-	+
18.	Subacute	-	+	+	+	+
19.	Chronic	+	+	+	+	-
20.	*Subacute	-	+	+	-	-
21.	Chronic	-	+	+	+	-

\* Contracted the disease in the European Theater of Operations.

NA=Records not available.

## DISCUSSION

This disease is one of varied manifestations. Admittedly the most easily recognizable signs are those concerning the skin. However, many other systems of the body have been seen to be involved. The altered function of the body as shown by the occasional finding of decreased serum protein and the lowering of the albumin-globulin ratio gives further evidence of more than a local involvement of the skin. Except for its relative benignity, the disease can perhaps be considered analogous to lupus which also has visceral as well as dermal manifestations. The liver and hematopoietic systems apparently are not infrequently involved. Possibly sufficient study would indicate some involvement in even the milder cases. The occurrence of hematopoietic disturbance, although infrequent, should be kept in mind.

All of the patients investigated, with one exception, came from the Southwest Pacific area. All in this series had had atabrine, including the patient who came from the European Theater of Operations. The amounts varied, but all of them had taken the drug for some time, either in suppressive or therapeutic doses, or both. As to malaria, complete records were not available. All patients came from regions where malaria is endemic. In 18 cases records were available. Of these, 9 gave a positive and 9 a negative history of malaria (Table III). It is of interest that 7 of the 21 patients had clinical relapses with parasites in their blood during their stay at Schick General Hospital (Table III). Atabrine has been mentioned as the determining factor. It still is not clear why this condition should occur proportionately much more frequently in New Guinea than other regions where similar conditions prevail. It is possible that atabrine is only one of the factors in a chain of causes which may eventually be found in diet, climate, ingested drugs, insecticides, or infection with malaria. It would seem advisable to evacuate the patient to the zone of the interior once the disease has become manifest.

#### CONCLUSIONS

1. The disease heretofore described as lichen planus (Southwest Pacific) is one of varied manifestations, the skin being only one of the sites of localization of lesions. It occurs only in those who have been taking atabrine.
2. Although occurring most frequently, by far, in the Southwest Pacific, it has also been found in the European Theater of Operations. One such case is included in this report.
3. The skin lesion can be described pathologically as occurring in three stages which merge into one another. It resembles other lichenoid dermatoses and is easily differentiated from them morphologically only in the acute stage.
4. Visceral complications, particularly hematologic and hepatic, are encountered. Severe aplastic anemia developed in 2 of the 21 patients in the series studied.

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[ *Illustrations follow* ]

## DESCRIPTION OF PLATE

### PLATE 102

FIG. 1. Acute stage. Hyperkeratosis. Marked cellular infiltrations of the papillae and corium. Edema and degeneration of the rete. Hematoxylin and eosin stain.  $\times 85$ .

FIG. 2. Subacute stage. Edema and degenerative changes are still seen in the stratum basalis. Cellular infiltrations have decreased in amount. Collections of pigment occur chiefly in chromatophores. Hematoxylin and eosin stain.  $\times 85$ .

FIG. 3. Chronic stage. Hyperkeratosis and plugging of hair follicles. Involvement of basal layer is slight. Scattered inflammatory cells remain, particularly around the hair shafts. Some chromatophores are seen. Hematoxylin and eosin stain.  $\times 85$ .

FIG. 4. Liver. Collections of chronic inflammatory cells and pigment-bearing macrophages in the liver parenchyma. Becker's stain for melanin.  $\times 285$ .



1



2



3



4





## COEXISTENT PULMONARY ASBESTOSIS AND SARCOIDOSIS \*

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Pulmonary asbestosis, regarded as a "modern disease" by Gloyne and Merewether,<sup>1</sup> was first described by Murray<sup>2</sup> in 1900. Although Fahr<sup>3</sup> described a case in 1914, interest in this disease was not re-awakened until the case of Cooke and McDonald was described in 1927.<sup>4</sup> Since that time, there have appeared in the available literature reports upon approximately 150 necropsies on cases of pulmonary asbestosis.<sup>5-12</sup> The paucity of proved cases, in comparison with those of silicosis, is not due wholly to failure to report such cases, for in large necropsy series asbestosis is apparently of infrequent occurrence.<sup>5-13</sup> Further, despite the widespread usage of asbestos products, there are comparatively few people engaged in the asbestos industry. As of October, 1944, only 19,700 people were employed in this industry in the United States.<sup>14</sup>

Much has been written about the clinical, roentgenologic, and biopsic aspects of sarcoidosis. However, because of the infrequency and relatively benign character of this disease there are only isolated detailed necropsy reports. From the available literature there have been found only 58 reports of necropsies on cases of sarcoidosis.<sup>15-21</sup> Most of these were summarized by Pinner.<sup>15</sup>

These two diseases present many clinical and roentgenographic similarities, and, also, their more frequent fatal complications are alike: pulmonary tuberculosis and cardiopulmonary insufficiency. Bronchogenic carcinoma, a frequent complication of pulmonary asbestosis, has not, however, been described as associated with sarcoidosis. Likewise, there has not been a previous description of asbestosis with coexistent sarcoidosis. It is the purpose of this report to present the findings in such a unique case, the only example of either pulmonary asbestosis or sarcoidosis in a series of 1870 necropsies done at this hospital.

### REPORT OF CASE

The patient was a white male, 42 years of age. Subsequent to hemorrhoidectomy in December, 1943, he had noticed that slight activity produced shortness of breath. He did not experience nocturnal dyspnea and he was able to lie flat in bed without respiratory difficulty. There was no history of cough, hemoptysis, or cardiac embarrassment. Notwithstanding a good appetite and the absence of gastric

\* Received for publication, May 9, 1945.

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symptoms, there was a weight loss of 22 pounds from December, 1943, to March, 1944, at which time he presented himself for medical care.

The patient had worked in an asbestos plant for 25 years, the last 10 years having been in a supervisory position. During this entire time he had worked in one department in which asbestos pipe was made. There was a slight but appreciable dust hazard associated with the sawing and splitting of the dried asbestos pipe, despite precautionary exhaust ventilation. The total time the patient had spent upon this final operation of sawing was unknown; nor was it learned whether he had been negligent in using the provided respirators. To the company's knowledge, this was their first case of asbestosis.

Physical examination revealed the following findings: Temperature, 37° C.; respiration, 22 per minute; arterial blood pressure, 105/70 mm. Hg; height, 170 cm.; weight, 67 kg. The chest was of increased anteroposterior diameter. Respiratory excursions were equal but decreased. The percussion note was resonant and auscultation revealed fine râles over the bases of the lungs, posteriorly. There were no evidences of cardiac enlargement, irregularity, or decompensation. Cyanosis and clubbing of the fingers were absent. The liver was barely palpable.

Report on the roentgenogram of the chest (Fig. 1) was as follows: "The bony framework is normal. The trachea is in the midline. The hilum shadows are moderately enlarged, bilaterally. One small calcified area is present in each hilum. There are numerous small nodular densities scattered throughout both lung fields, especially throughout the lower lobes. There is some confluence of these densities in the left lower lobe. Emphysema is present."

Examination of the blood showed erythrocytes, 5.5 million; leukocytes, 6.6 thousand; 73 per cent neutrocytes; 21 per cent lymphocytes; 5 per cent monocytes; 1 per cent eosinophils; sedimentation rate, 26 mm. No abnormality was found in the urine. The vital capacity, 2200 cc., was 51 per cent of normal. Tuberculin tests were not done.

The patient was seen at regular intervals and his only complaint was increasingly severe exertional dyspnea. A roentgenogram of the chest 4 months after the initial chest film revealed no new findings. Although the patient greatly limited his activities, dyspnea became progressively more severe so that eventually, even at bed rest, there was extreme air hunger. At no time were there evidences of cardiac failure. He died approximately 11 months after the onset of symptoms, apparently from respiratory failure.

### *Autopsy Findings*

The necropsy was performed 5 hours after death. Superficially, there was considerable decrease in the subcutaneous tissues and the body musculature. There was no clubbing of the nailbeds or dependent edema. The mediastinum was in the midline. Each lung completely filled its hemithorax and extended far into the anterior mediastinal space. The domes of the diaphragm, anteriorly, were at the level of the fourth interspace and fifth rib, right and left respectively.

The lungs were encased in markedly thickened, tough, yellowish white, generally fused pleurae. The interlobar fissures were obliterated by easily broken adhesions. Lobation was normal. Hemorrhagic fibrinous material, present over the posterolateral aspect of the left lower lobe, loosely bound the thickened parietal pleura to the lung in this area.

The frontal section of the left lung (Fig. 3) revealed coarse, lacy, tannish brown, hypercrepitant tissue throughout both lobes. Innumerable slightly elevated, grayish green, irregular, firm nodules, 1 to 2 mm. in diameter, were present throughout the lung. Thin, radiating, fibrous bands surrounded and connected these nodules. Also, slightly thickened pleural septa extended into the lung substance for variable depths. In the lung tissue about the bronchi of the second and third interspaces these nodules were somewhat confluent and a similar change was noted in the subpleural tissues for a depth of 3 to 5 mm. Generally, these nodules, present in moderate numbers, were separated by wide zones of dry emphysematous lung tissue studded by numerous minute, grayish tubercles. Dissection of the bronchi of the lower lobe disclosed that they were moderately dilated, cylindrically and saccularly, and lined by glistening white mucosa.

In the right lung the same changes were observed as were present throughout the left. However, the grayish green, irregular nodules tended to be more numerous, larger, and more confluent. This was particularly true of the anterior portions of the lower and middle lobes. Also, the nodules were connected by thicker grayish black and grayish white interlacing bands of fibrous tissue. Extending deeply into the lung substance, thickened pleural septa communicated with the fibrous tissue in and about the clusters of tubercles. Except for more pronounced dilatation and thinning of the mucosa, the bronchi of the right lung were similar to those of the left.

The tracheobronchial lymph nodes were moderately enlarged and on section consisted of dense, rubbery, anthracotic centers and thin rims of yellowish white tissue. Calcification was not grossly demonstrable.

The embalmed heart weighed 280 gm. and had the following measurements: tricuspid valve, 120 mm.; pulmonary valve, 80 mm.; mitral valve, 85 mm.; aortic valve, 65 mm.; right ventricle wall, 3 to 8 mm.; left ventricle wall, 15 mm. The greatest transverse cardiac diameter was 13.5 cm. (The estimated normal heart weight on the basis of body length is 317 gm., plus or minus 40.<sup>22</sup>) The tricuspid/aortic valve and pulmonic/aortic valve ratios were 1.84 and 1.23, respectively. (These normally should be 1.68 and 1.05, respectively.<sup>23</sup>) The right ventricle was dilated and its columnae carneae and papillary muscles were more prominent than usual. No mural thrombi were demonstrable and the valvular endocardium was normal.

The enlarged spleen was of normal configuration and measured 18 by 9 by 6 cm. It was covered by a smooth capsule and the splenic

substance was firm and purplish red with normal markings. No tubercles were seen. The liver measured 22 by 16 by 10 cm. The remaining organs showed passive hyperemia and moderate generalized arteriosclerosis.

### *Microscopic Findings*

Throughout the lung there was a conspicuous linear, interlacing, peribronchial and septal pulmonary fibrosis (Fig. 2). This was particularly prominent in the subpleural tissues. The intervening lung tissue was moderately emphysematous. Innumerable tubercles were present in the linear and peribronchial fibrotic areas and were present to a lesser extent in the walls of the respiratory bronchioles and the adjacent alveolar walls. Generally, these tubercles were of two types: sarcoidal and foreign body granulomas. The former predominated by approximately ten to one. Tubercles of these types were intimately associated and, in addition, many intermediate types were presented.

The sarcoidal tubercles (Fig. 4) were free of caseation, contained no demonstrable organisms and were, for the most part, in the same stage of development; however, a minimal number presented some peripheral fibrosis and there was an occasional, coarse, collagenous ball. Generally, the tubercles were sharply demarcated, surrounded by delicate reticulum, and did not present peripheral rims of lymphocytes. They consisted of peripherally arranged epithelioid cells surrounding central, loosely arranged epithelioid and monocyctic cells. Giant cells were, for the most part, centrally located and often comprised over half of the bulk of the nodule. The giant cells appeared to be of two types: Langhans' cells and foreign body giant cells, with the former predominating. In many of the Langhans' cells there were numerous small vacuoles, each containing a pink, round body. Other Langhans' cells contained large, clear vacuoles; and, rarely, in those cells containing one large vacuole there was present an "asteroid" body, an intensely eosinophilic stellate mass, 15 to 20  $\mu$  in diameter (Fig. 5).

More frequently, the Langhans' cells contained round, oval, or suggestively budding, intracytoplasmic bodies of Schaumann,\* 25 to 50  $\mu$  in diameter. Rarely, these bodies appeared to lie outside of giant cells, and some enclosed irregular yellowish material (Figs. 6 and 7). These bodies stained blue with hematoxylin and in ferrocyanide preparations were strongly positive for iron. Dr. Leroy U. Gardner,<sup>24</sup> who also studied this case, stated that these bodies stained "red with acid fuchsin of van Gieson-Weigert instead of black like elastic tissue"

\* Schaumann, J. On the nature of certain peculiar corpuscles present in tissue of lymphogranulomatosis benigna. *Acta med. Scandinav.*, 1941, 106, 239-253.

and that "von Kossa's calcium stain is negative." Re-study of appropriately stained sections revealed, as pointed out by Gardner, that the Schaumann bodies did stain red; however, a moderate number also contained calcium in variable degrees, as demonstrated by von Kossa's stain. An occasional giant cell contained one or more clefts suggestive of cholesterol crystals. More frequently, however, doubly refractile, irregular spicules, plaques, and conchoidal masses were observed in giant cells. These doubly refractile masses were often about, or in, the Schaumann bodies, particularly the smaller and partially calcified forms.

The foreign body tubercles were indefinitely demarcated and consisted of rather closely packed, indefinitely arranged, large monocytes, and one or more foreign body giant cells. These tubercles, for the most part, were within the dense zones of fibrosis. Some, however, were present in alveoli and respiratory bronchioles. Golden yellow discoid, verruciform, and incompletely segmented asbestos bodies, many of which were in giant cells, were observed in and about the nodules (Fig. 8). Asbestos bodies, singly or in clusters and in moderate numbers, were present also in the dense fibrotic areas (Fig. 9) and occasionally within alveoli (Fig. 10). Rare, laminated, calcified masses, enclosing apparent asbestos bodies (Fig. 11) and other bodies which appeared to be of the Schaumann variety, were present in the linear fibrotic bands. Asbestos bodies were also encountered in and about the sarcoidal tubercles and in the associated sarcoidal giant cells of both varieties, but more frequently in those of foreign body type. Iron preparations clearly demonstrated the bizarre forms of the asbestos bodies.

In many areas it was difficult to distinguish between the two types of lesions. This was particularly true throughout the subpleural region where both the lesions and asbestos bodies were more numerous, clustered, and embedded in a dense matrix of collagen, masses of coarse elastic fibers, and fine reticulum.

The larger bronchi were remarkable only for slight chronic inflammation. The bronchioles and respiratory bronchioles, embedded in dense collagen and surrounded by tubercles, were moderately dilated and presented conspicuous focal squamous metaplasia and moderate chronic submucosal inflammation. In the subpleural regions where the asbestotic fibrosis and the granulomatous reaction were most intense, the bronchioles were irregularly dilated and lined by alternating strips of tall columnar and squamous epithelium. Only a few bronchioles contained neutrocytic exudate. The respiratory bronchioles were generally constricted, surrounded by masses of elastica, and

many contained asbestos bodies and the associated granulomatous reaction. Within the peribronchial fibrous tissue there was a moderate amount of hemosiderin in linearly disposed granules, and fine lipoid droplets. The small pulmonary arteries and arterioles presented slight intimal thickening, and those in the subpleural zone were surrounded by thick collars of elastic fibers.

The intervening alveoli were moderately dilated, the capillaries were congested, and there was a slight increase in collagen in the alveolar walls bordering the fibrous masses. Focally, clusters of alveoli contained lipoid-laden macrophages. "Heart lesion cells" were infrequent.

Sections of the pleura revealed dense, laminated, and oval fenestrated bundles of collagen. Focally, there were indefinitely demarcated nodules which consisted of circularly disposed lamellae of collagen. Superficially, the pleura presented slight fibroblastic activity and an occasional perivascular accumulation of lymphocytes and monocytes, some of the latter occasionally containing hemosiderin. No asbestos bodies were observed. The pleura over the left lower lobe, in addition, bore organizing fibrinous exudate on its visceral aspect.

Sections of the tracheobronchial lymph nodes presented a repetitious pattern of sarcoidal tubercles with almost complete replacement of the lymphoid tissue. Throughout the nodes there were minimal diffuse fibrosis and several nodular masses of coarse collagen. The tubercles were similar to those in the lung as to structure and stage of development. Inclusions of Schaumann were not observed and only a rare "asteroid" was present. Asbestos bodies were not identified. A moderate number of hemosiderin-containing macrophages were present in the remaining lymphoid tissue.

Similar sarcoidal tubercles were present to a slight degree in the spleen and liver, and to a lesser extent in the kidneys, diaphragmatic muscle, and the right and left ventricular myocardium. These sarcoidal tubercles, however, were not as compactly arranged as those in the lung and tracheobronchial lymph nodes, and were surrounded by and permeated by lymphocytes. "Asteroid bodies" and Schaumann bodies were not present in the giant cells of these tubercles. No asbestos bodies were found. Those in the right ventricular myocardium were associated with considerable fibrosis.

The results of chemical and spectrographic analysis of lung tissue, performed under the direction of Dr. Leroy U. Gardner,<sup>24</sup> are presented in Table I.

The final diagnoses were: Moderate pulmonary asbestosis; extensive sarcoidosis of pulmonary and tracheobronchial lymph nodes; marked chronic pulmonary emphysema; slight sclerosis of the small

arteries and arterioles in the lungs; marked nodular obliterative pleural fibrosis; focal organizing fibrinous pleuritis; minimal sarcoidosis of the heart, liver, spleen, and kidneys; right ventricular cardiac dilatation and relative right ventricular hypertrophy; acute passive hyperemia of the viscera; slight cirrhosis of the liver; slight generalized arteriosclerosis; minimal focal chronic adrenalitis and nephritis; chronic posterior urethritis and interstitial prostatitis.

TABLE I

*Chemical and Spectrographic Analysis of Ash*  
(Dry Tissue, Approximately 14.1% of Moist Tissue. Ash, 6.70% of Dry Tissue.)

As oxides (except Cl)		As elements		
Chemical analysis			Chemical analysis	Spectrographic analysis
	Per cent		Per cent	Arbitrary scale of relative amounts
Cu, Ag, Hg } Pb, Bi, Cd } Mo	<0.15	Na	4	75
SiO <sub>2</sub>	2.76	K	36.7	100
Fe <sub>2</sub> O <sub>3</sub>	8.03	Sr	None found	1.5
Al <sub>2</sub> O <sub>3</sub>	0.37	Ba	None found	5
BeO	None found	Ca	2.1	80
ZnO	0.39	Al	0.2	50
MnO	0.03	Mg	0.7	75
CaO	2.92	P	8.0	60
MgO	1.18	Si	1.3	100
BaO	None found	Fe	5.6	75
SrO	None found	Mn	0.02	3
TiO <sub>2</sub>	None found	Ti	None found	5
V <sub>2</sub> O <sub>5</sub>	None found	Cu		25
Cr <sub>2</sub> O <sub>3</sub>	0.07	Ag		2
NiO, CoO	<0.05	Sn		3
Na <sub>2</sub> O	5.44	Cr	0.05	3
K <sub>2</sub> O	44.40	B		1
P <sub>2</sub> O <sub>5</sub>	18.42	Be	None found	0
Cl	4.61	Pb		25
CO <sub>2</sub>	Present	Zn	0.3	5
		Bi		10
		Pt		3
		Cl	4.6	0
Total	88.82			

## DISCUSSION

Clinically, in view of the significant history of exposure to asbestos, the possibility of sarcoidosis was never entertained. In retrospect, the rapidly progressive, disabling dyspnea, unaccompanied by evidences of enlargement of the right heart or cardiac failure, should have aroused suspicion that there was a concomitant pulmonary lesion. Asbestosis alone is not usually accompanied by such profound, rapidly developing, respiratory embarrassment. In this case, however, there were no collateral clinical evidences of sarcoidosis. It would seem that a clinical diagnosis of coexistent asbestosis and sarcoidosis would be justified only by biopsy of a lymph node or a skin lesion to demon-



strate sarcoid lesions and the discovery of asbestos fibers in the sputum, with a history of adequate exposure to asbestos fibers and roentgenographic evidences of diffuse pulmonary fibrosis. Asbestosis of the degree observed, alone should not have caused death, and sarcoidosis has generally been regarded as a benign process. Reisner,<sup>18</sup> however, on the basis of his observations on cases of pulmonary sarcoidosis, stated "that one is not justified in assuming too confident an attitude regarding the ultimate outcome." This statement is particularly true when, as in this case, sarcoidosis complicates pre-existing pulmonary disease.

Pathologically, there were evidences of right heart strain in that there was marked dilatation of the right heart, evidenced by increased tricuspid and pulmonic/aortic valve ratios and slight passive hyperemia of the viscera. The total heart weight, however, on the basis of body length,<sup>22</sup> was normal. As determined by the ratio of the left and right ventricular weights, it has been shown that there may be considerable relative right ventricular hypertrophy without an increase in the total heart weight. However, relative right ventricular cardiac hypertrophy in Higgins' series<sup>25</sup> was not usually accompanied by evidences of right ventricular failure. In view of the significant dilatation of the right side of the heart and the slight sclerosis of the pulmonary arterioles, there was, in all probability, some degree of pulmonary hypertension in this case. However, in the absence of an increase in total heart weight and in the absence of evidences of chronic passive hyperemia of the viscera there was probably no, or insignificant, exaggeration of air hunger due to heart failure.

It has been suggested that dyspnea in the pneumoconioses is due to capillary and arterial blockage by the fibrotic process. This, in all probability, is true to a variable degree in those persons with severe fibrosis of the conglomerate type with attendant extreme chronic emphysema. This hypothesis, however, does not explain the severe dyspnea that is seen in occasional cases of diffuse miliary studding of the framework of the lung by silicotic, tuberculous, sarcoidal, or neoplastic tubercles. It may be that the mechanism of dyspnea in such instances is due to irritation of the vagus nerve endings with reflex stimulation of the respiratory center (Hering-Breuer reflex). In view of the equivocal evidences of hypertrophy of the right heart in this case, mechanical obstruction to the blood flow would not appear to be the responsible factor but, more likely, because of the diffuse active inflammatory process throughout the lungs, the Hering-Breuer reflex was exaggerated. Presumably, there was either a severe re-

spiratory alkalosis or acidosis. Tissue changes suggestive of alkalosis, such as calcification of the renal tubules, were not found.

Microscopically, there was some difficulty in differentiating the two types of tubercles since there were many sarcoidal tubercles which contained asbestos fibers, and tubercles of indeterminate type, not containing fibers or inclusion bodies, were sometimes seen. It was difficult to determine how much of the fibrosis was due to asbestosis. Morphologically, since the majority, by far, of the sarcoidal tubercles were without evidences of fibrosis and apparently of the same age, it is suggested that this process was engrafted upon an established asbestosis. Further, on the basis of Gardner and Cummings' <sup>26</sup> experimental studies on asbestosis, the marked peribronchiolar fibrosis with sequestrated asbestos bodies, the marked pleural fibrosis and pleural septal fibrosis, and the metaplasia of the bronchiolar epithelium indicate that the asbestosis was well established and over 700 to 800 days old. Dr. Leroy U. Gardner, who kindly examined the material, stated: "In comparison with our other material the pigmented foci in your case seem to show more fibrosis and less localized emphysema. Histologically, this can probably be explained by the presence of sarcoid nodules within the asbestotic zones of reaction. I would infer that in your case the two conditions developed more or less simultaneously, but that probably the asbestosis was present to some degree before the sarcoid appeared. This opinion is based upon the occurrence of asbestos fibers and other iron-containing particles in the interior of the tubercle-like nodules and in some cases within the giant cells themselves. The number of asbestos bodies is smaller than seen in many cases."

Inclusions of the Schaumann variety, found only in the lung, occurred in 4 per cent of the giant cells. Some of these enclosed golden-yellow, irregular bodies suggesting asbestos bodies, but similar to organic material previously described within such bodies. Yet there were definite asbestos bodies enclosed by similar dark blue material. Schaumann inclusions have been described in only 4 per cent of the reported necropsies on sarcoidosis as summarized by Rubin and Pinner, <sup>17</sup> who did not regard these inclusions as specific for sarcoidosis. Rich, <sup>27</sup> who was impressed by the frequency of Schaumann inclusions in sarcoidal lesions and by their absence in unequivocal tuberculous lesions, noted that Metchnikoff reported the presence of calcified inclusions in the hyperplastic tuberculous lesions of experimentally infected Algerian rats. Kraus <sup>20</sup> stated that the presence of calcified inclusions was a feature not found in any known granuloma except

sarcoidosis. Gardner<sup>24</sup> pointed out that, in his sarcoid material, these bodies, regarded by many to consist of calcium or calcified remnants of elastica, do not, by the von Kossa method, contain calcium, but, by the ferrocyanide method, give a strong reaction for iron. Only a moderate number of the Schaumann bodies observed in the present case were either wholly or partially calcified, yet all gave a strong reaction for iron. Studies on sarcoid lesions of lymph nodes and spleen from another case revealed only a few iron-staining noncalcified Schaumann bodies. The presence of doubly refractile, nonlipoid substance in giant cells and frequently in close relation to Schaumann bodies has not been emphasized in the literature on sarcoidosis. It has been noted, however, that colorless and yellowish tinged refractile material is often enclosed by the Schaumann body. The fact that these masses are frequently doubly refractile has not been stressed. It has been suggested that these enclosed masses represent disintegrating elastica; however, van Gieson-Weigert stains do not confirm this suggestion. The origin of this refractile and doubly refractile material is not known. Being in and about many of the small, partially calcified bodies, this doubly refractile material appears to be associated with the development of the Schaumann body. The larger and more densely stained bodies were not as frequently associated with visible doubly refractile substance. However, fractured and fragmented, apparently old, Schaumann bodies, as seen in control sarcoid material from lymph node and spleen, usually contained moderate amounts of doubly-refractile substance. Apparently then, the Schaumann body, which stains blue with hematoxylin and red with acid fuchsin, is formed in response to doubly refractile, nonlipoid substance and initially is impregnated by iron and later, in amounts demonstrable by von Kossa's stain, by calcium.

Wolbach,<sup>21</sup> in 1911, Jadassohn, in 1919,<sup>28</sup> and Friedman,<sup>19</sup> in 1944, have described a peculiar intracellular body in cases of sarcoidosis. This body, stellate in shape, varies in size up to 25  $\mu$ , generally lies in an intracytoplasmic giant cell vacuole, and stains intensely with acidophilic stains except the central area which is basophilic. Wolbach described them as lying free in tissue spaces, in endothelial leukocytes, and in giant cells. Friedman found such bodies in only 6 to 8 per cent of the giant cells in his case. Both investigators attempted to determine the chemical structure of this stellate body by specific stains; however, they were unsuccessful. Both considered the possibility of its being an extraneous organism, although questionable. Wolbach regarded it as a nonspecific biochemical alteration of the cytoplasm. He was never able to demonstrate stellate bodies in other

material and decided that they were not similar to inclusions sometimes seen in cases of sarcoma. Friedman regarded these bodies as nonspecific but highly characteristic of sarcoid lesions. Friedman proposed that these bodies be called "asteroids," but perhaps it would be better, eponymically, to call them Wolbach's asteroids. They have been described in 7 cases of sarcoidosis, and never in association with the Schaumann calcified inclusion body. In the present case asteroids were present in approximately 1 per cent of the giant cells in the lungs and tracheobronchial lymph nodes. Definite transition stages of asteroid formation were suggested by the presence of spicules on the pink, coccoid, intravacuolar, intracytoplasmic bodies, particularly in those giant cells in which the small vacuoles were clustered and disintegrating. In addition, an occasional Wolbach's asteroid, instead of lying in a large, clear vacuole, was surrounded by agminated ruptured vacuoles. In view of the presence of similar pink, coccoid, intravacuolar bodies, similar asteroids, and the same suggestive stages of asteroid formation in the giant cells of talcum powder granuloma, as observed in one case in this laboratory, these giant cell cytoplasmic changes must be regarded as Wolbach originally suggested, nonspecific biochemical cytoplasmic alterations. In addition, such an asteroid is depicted in the giant cells of leprous lesions by Mallory<sup>29</sup> who called them "spiculated" bodies. No transition stages between Wolbach's asteroids and Schaumann's inclusions were even remotely suggested.

The pathogenetic relationships of asbestosis and sarcoidosis are dependent upon the chronologic development of the lesions and the nature of the causative agents. Historically and histologically, in this case, it is most likely that asbestosis preceded the development of sarcoidosis. The predominant localization of the sarcoidal tubercles within asbestotic zones of fibrosis with attendant morphologic modification of both lesions, as evidenced by asbestos bodies within sarcoidal tubercles and lesions of indeterminate type, would suggest an analogy to the intimate relationship existent between tuberculosis and the pneumoconioses. It must be remembered, though, that even in uncomplicated sarcoidosis the lesions occur in the framework of the lung, and therefore the morphologic relationships of the two may be coincidental. This would be in agreement with those who believe that morphologically sarcoid is not reconcilable with tuberculosis. However, to those who regard sarcoidosis as a peculiar form of tuberculosis, this case then would be one of asbestosis with superimposed non-caseating tuberculosis.

The authors are indebted to Dr. Leroy U. Gardner who critically examined the gross and microscopic material of this case and supplied the chemical analysis.

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[ Illustrations follow ]

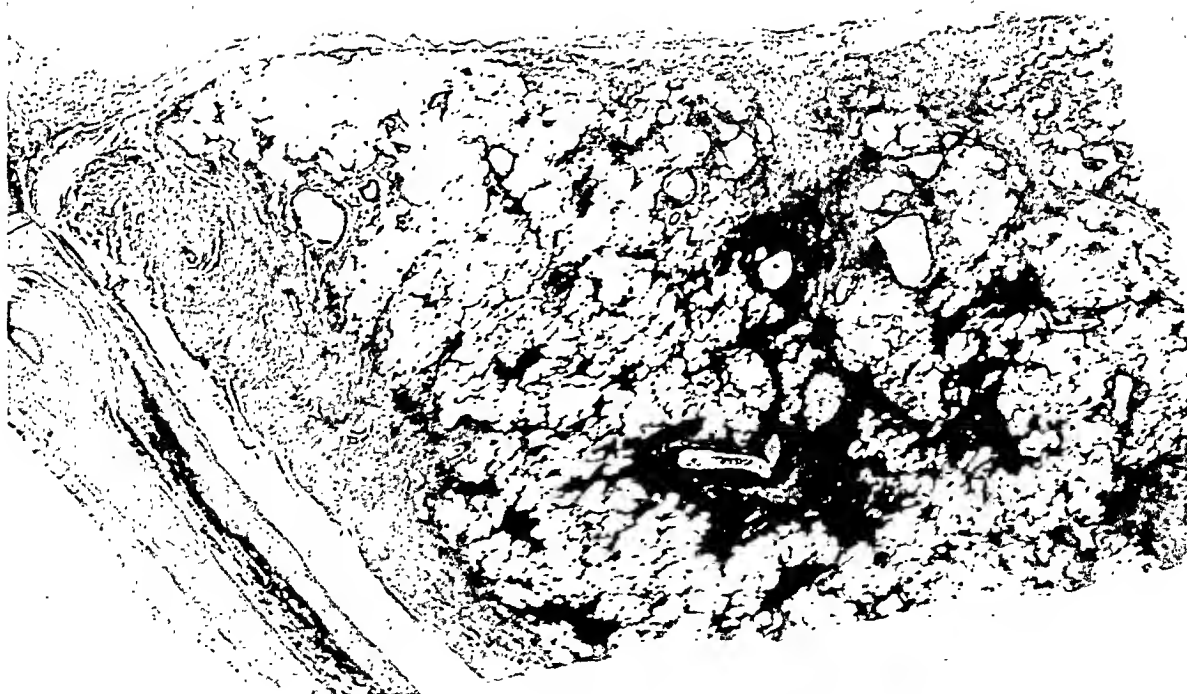
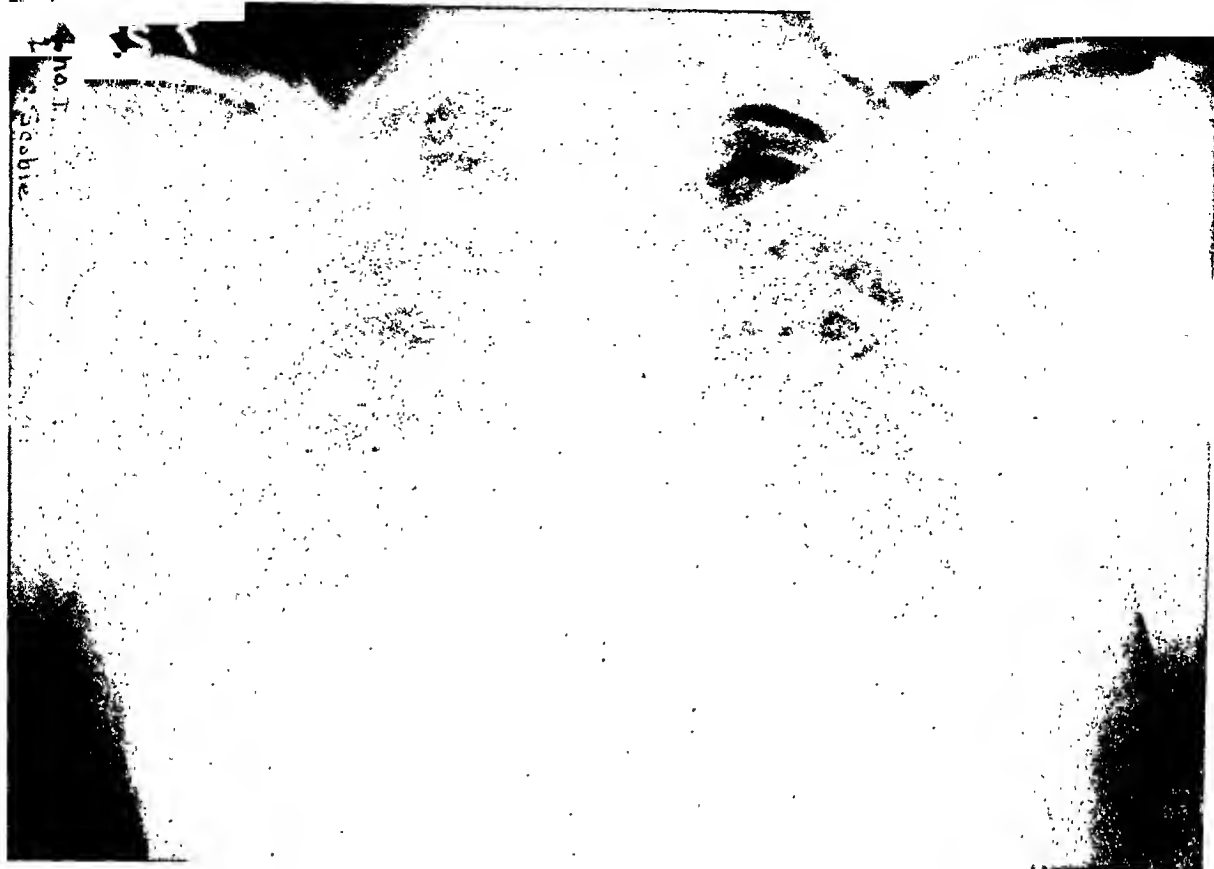
## DESCRIPTION OF PLATES

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### PLATE 103

FIG. 1. Initial roentgenogram of the chest.

FIG. 2. Photomicrograph of lung and adherent pleura showing subpleural, septal, peribronchiolar, and marked and focally nodular pleural fibrosis.  $\times 4$ .



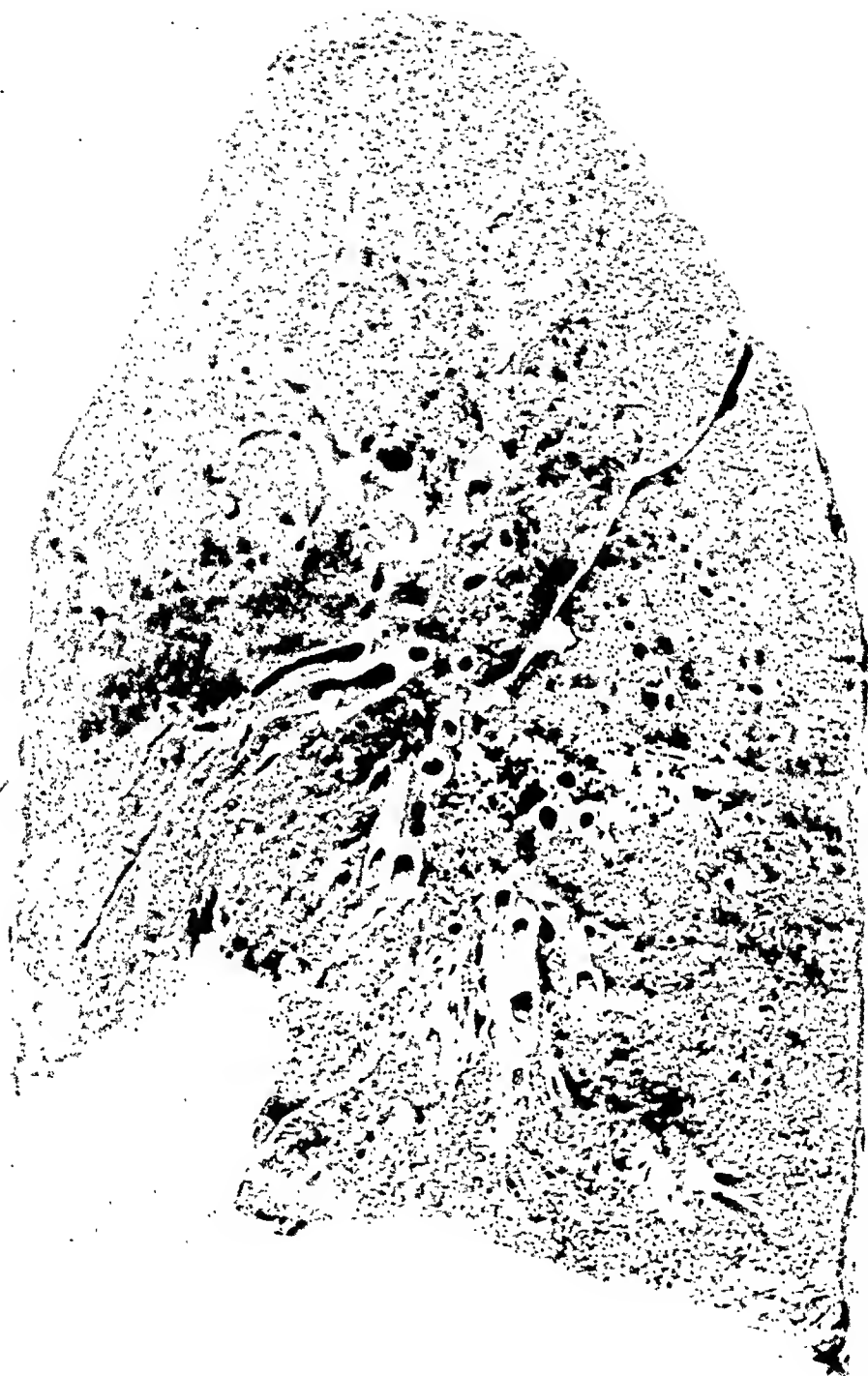
Skavlem and Ritterhoff

### Coexistent Asbestosis and Sarcoidosis



PLATE 104

FIG. 3. Frontal section of left lung.



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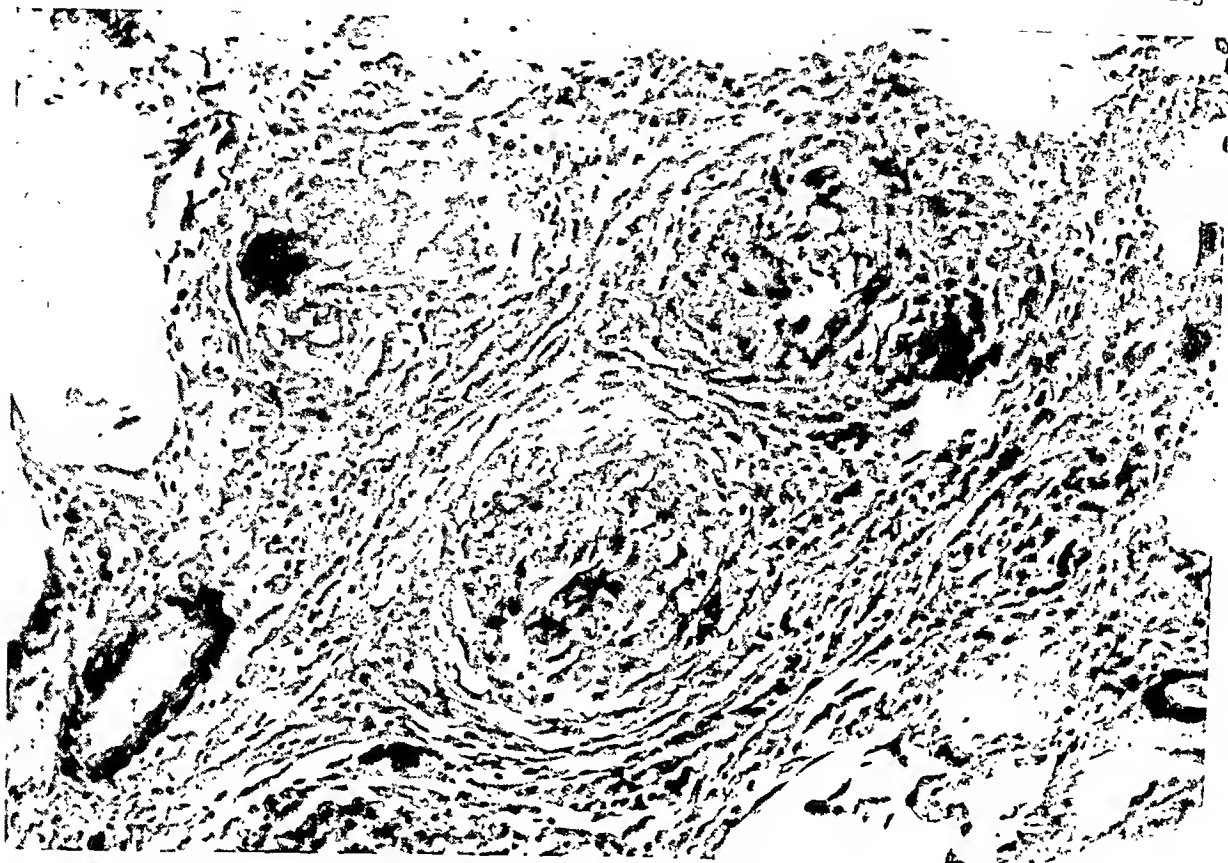
Coexistent Asbestosis and Sarcoidosis

PLATE 105

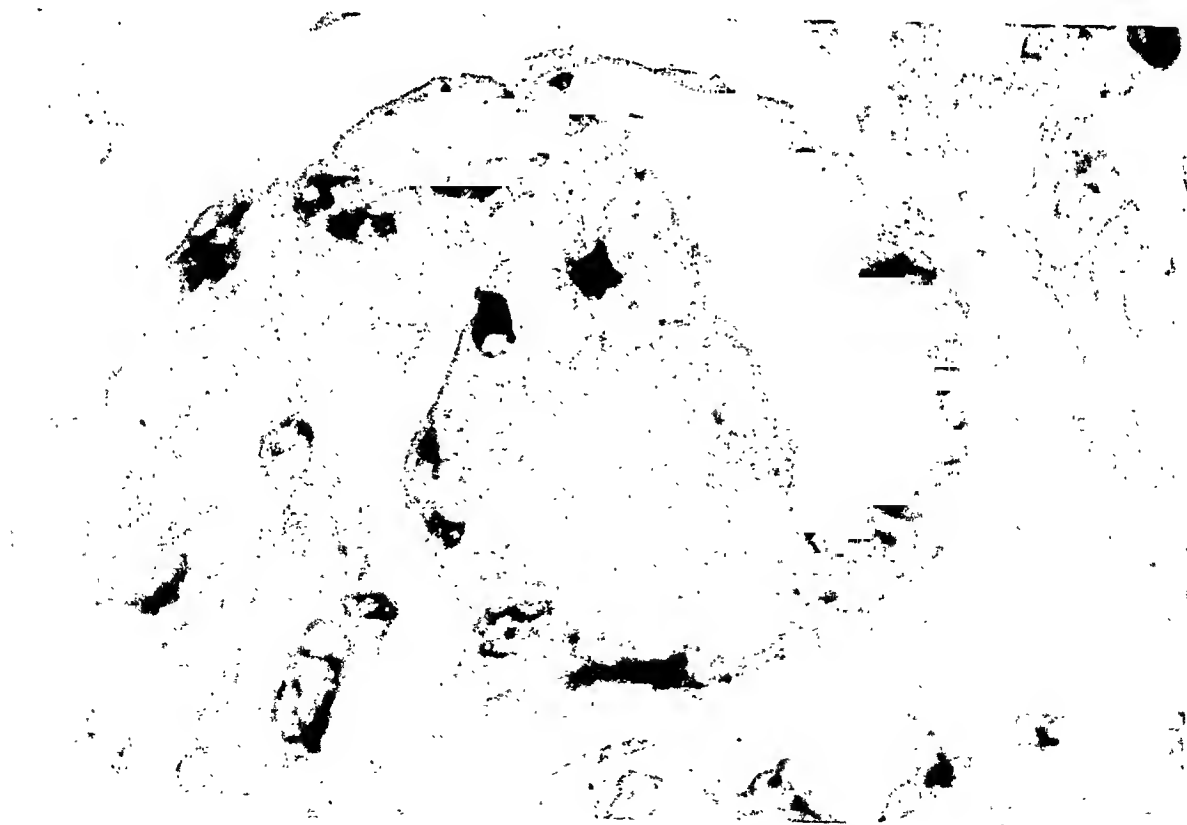
FIG. 4. Lung, showing a cluster of sarcoidal tubercles.  $\times 160$ .

FIG. 5. Lung. The giant cell which nearly fills the field contains an "asteroid" in an intracytoplasmic vacuole.  $\times 1090$ .

4



5



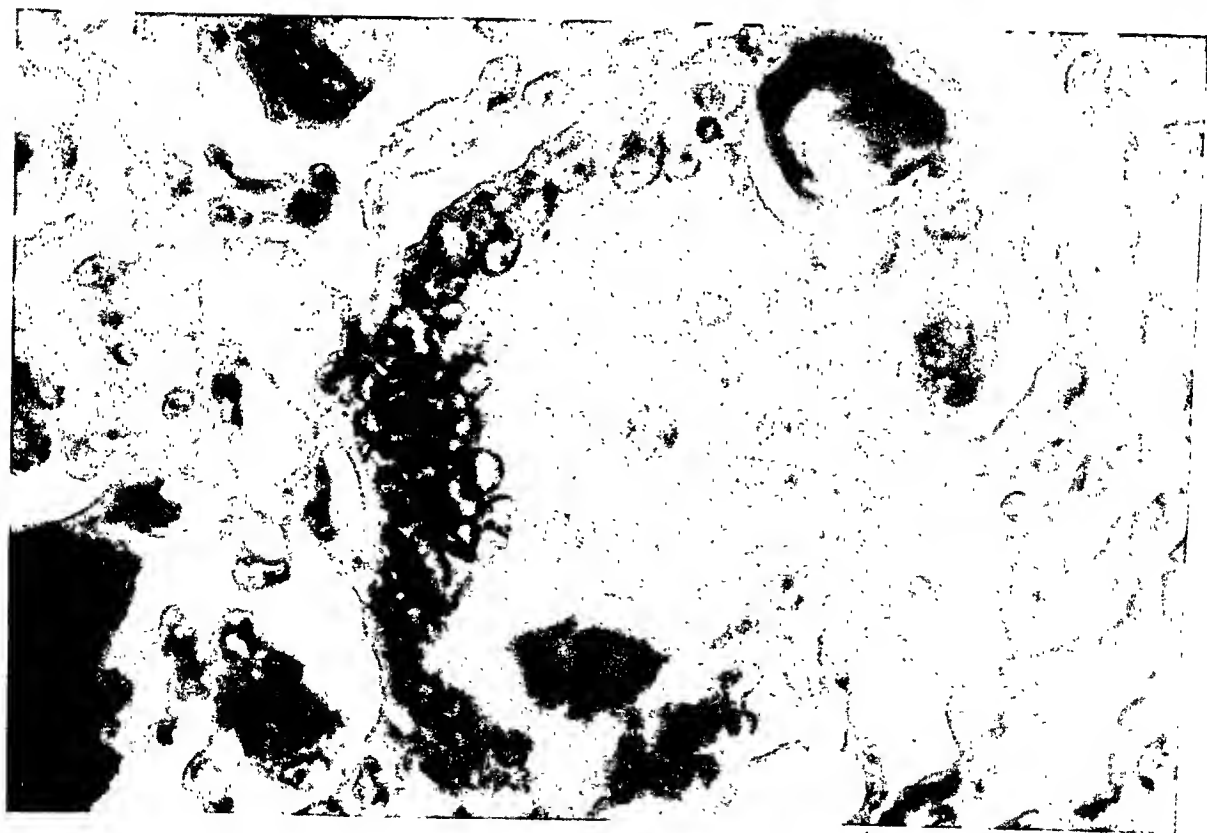
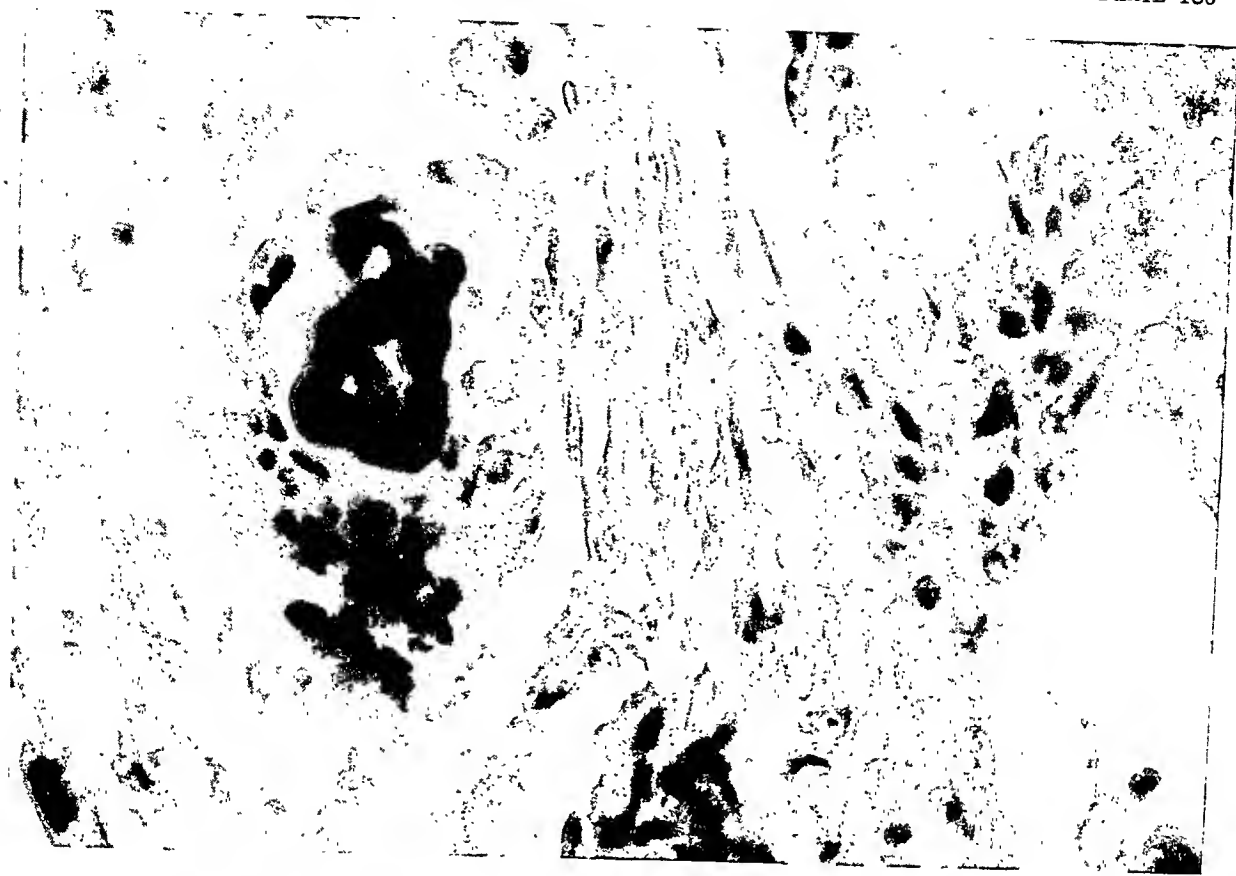
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Coexistent Asbestosis and Sarcoidosis

PLATE 106

FIG. 6. Lung. An inclusion of Schaumann encloses an oval yellow body. With polarized light, doubly refractile material surrounds this calcified mass.  $\times 725$ .

FIG. 7. A giant cell from the lung with inclusions of Schaumann.  $\times 725$ .



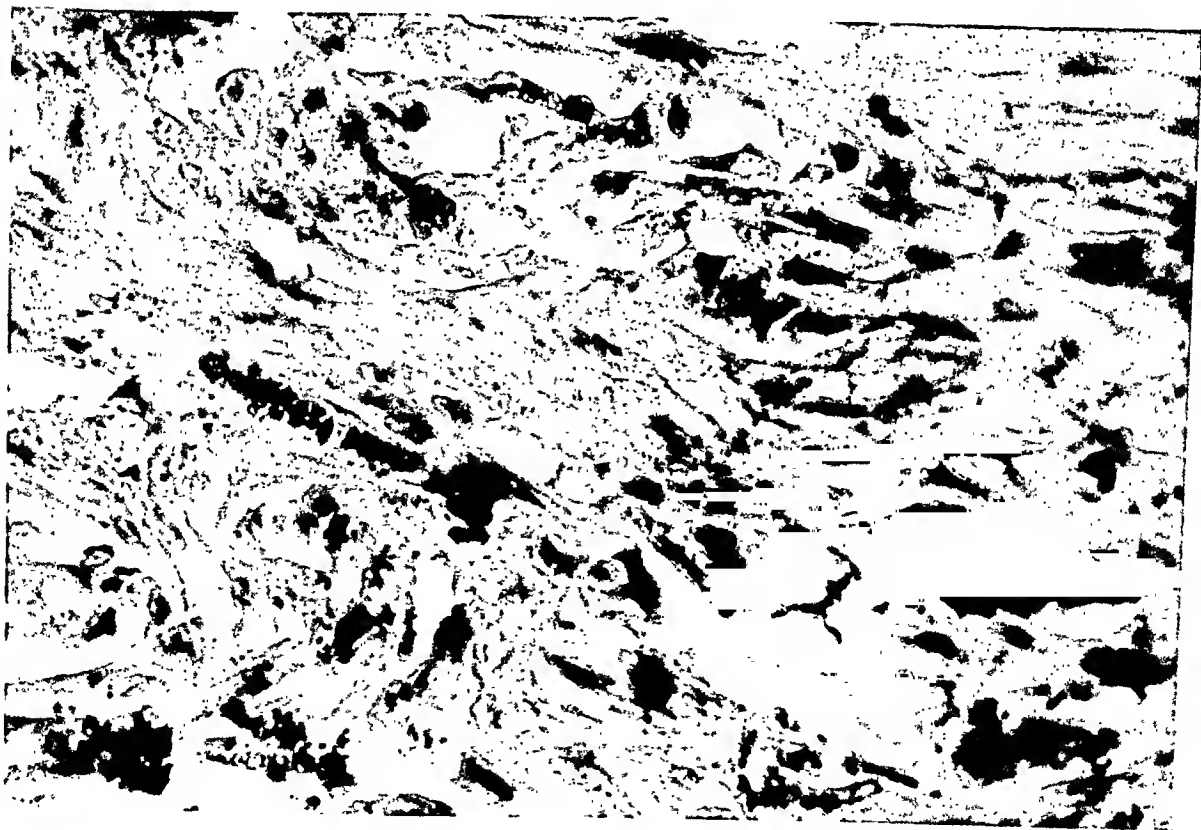
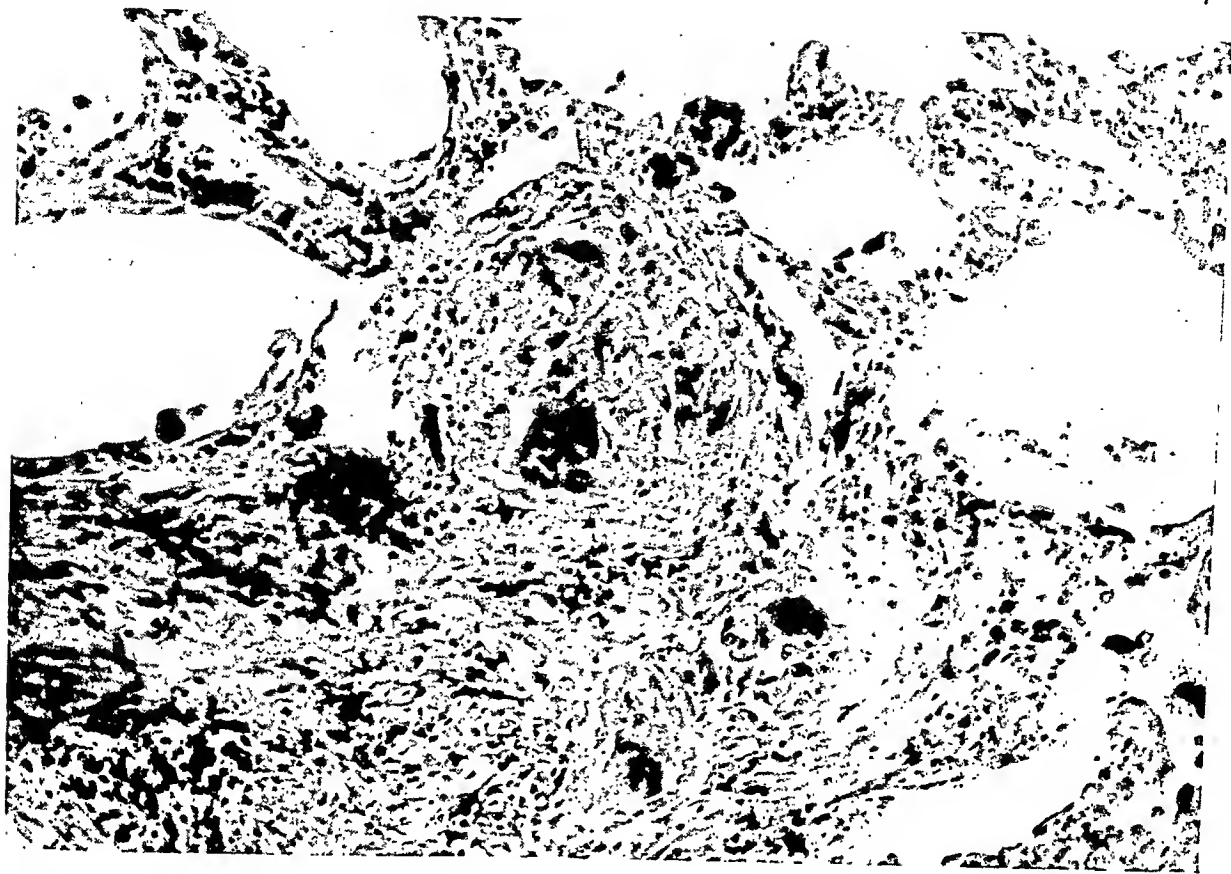
Skavlem and Ritterhoff

Coexistent Asbestosis and Sarcoidosis

PLATE 107

FIG. 8. Lung with tubercles of foreign body type. The central tubercle has an asbestos body at its periphery.  $\times 160$ .

FIG. 9. Lung showing asbestos bodies and clusters of hemosiderin-laden macrophages within an area of fibrosis.  $\times 725$ .



Skavlem and Ritterhoff

Coexistent Asbestosis and Sarcoidosis

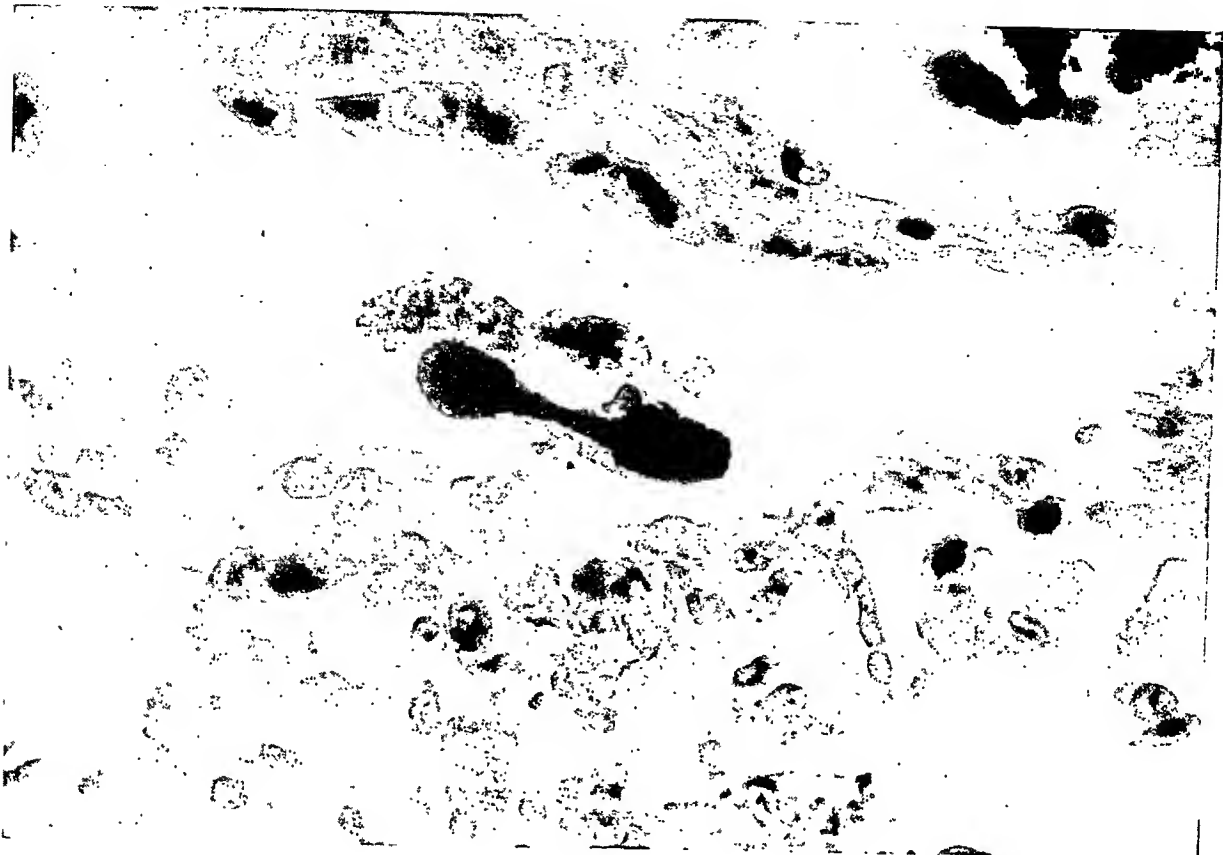


PLATE 108

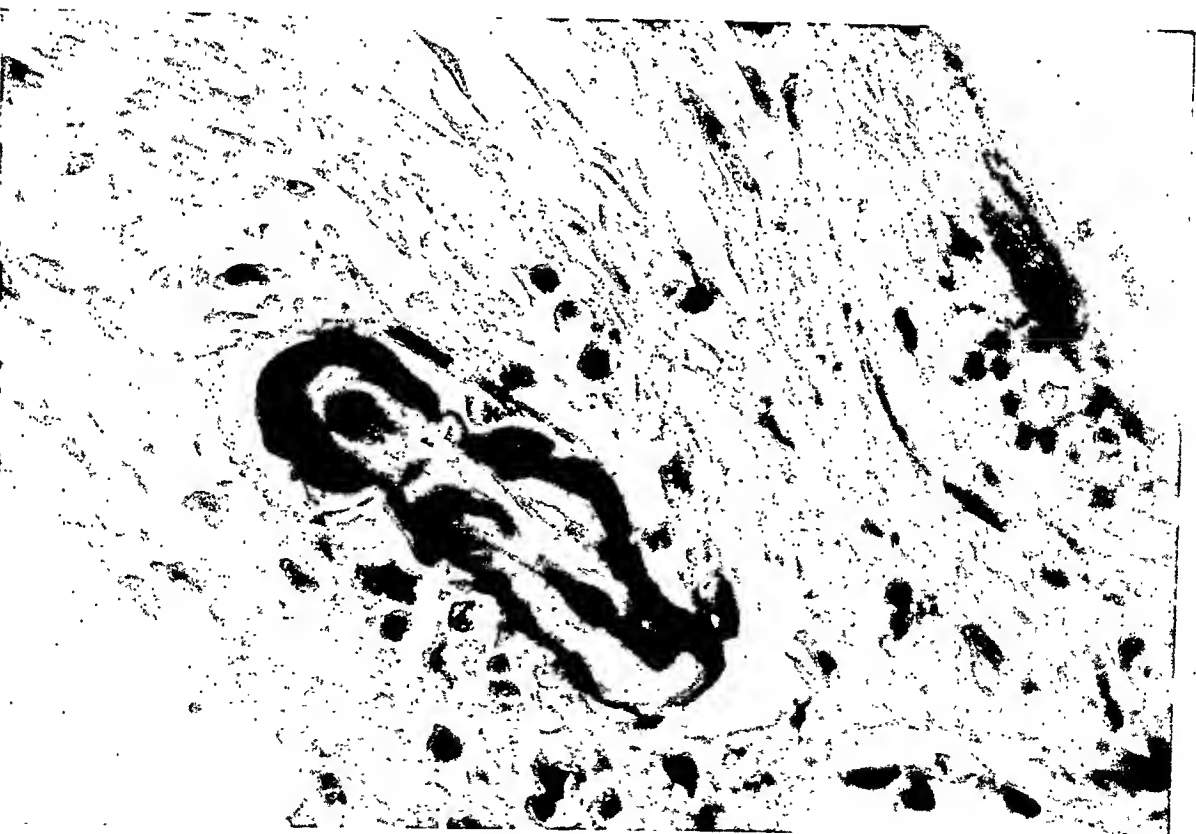
FIG. 10. Lung. An asbestos body is shown in an alveolus.  $\times 725$ .

FIG. 11. Lung showing an asbestos body encrusted with iron and calcium.  $\times 725$ .

□



1



Skavlem and Ritterhoff

Coexistent Asbestosis and Sarcoidosis



# XANTHOMATOSIS OF THE ARTERIAL MEDIA IN A DOG \*

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At a recent routine necropsy a dog was found to have xanthoma-like lesions affecting the media of the muscular arteries and some of the elastic arteries. In many of these vessels, fibrous connective tissue proliferation and deposition of iron-containing material were associated with lipid deposit and foam cell formation. In the few reported cases of vascular involvement in human xanthomatosis, the lesions were confined to the intima, and a primary, xanthoma-like, medial localization has never been described in any species, either spontaneously or following experimental procedures.

The medial lesions in this case differ considerably from the usual descriptions of spontaneous canine arterial diseases. In the most common of these there occur nodular fibrous intimal thickenings that affect principally the abdominal aorta. Fatty changes in these nodules are uncommon and primary deposits of fat in the otherwise uninvolved vessel wall are practically unknown. Medial calcification histologically similar to Mönckeberg's sclerosis is not unusual and predominantly involves the aortic arch in the vicinity of the aortic valves. Morehead and Little<sup>1</sup> have recently described, in addition, such spontaneous aortic lesions as focal elastic tissue loss with "grouping" of smooth muscle cells and medial necrosis with cyst formation. Multiple calcium deposits in the vascular walls as well as in the myocardium, endocardium, kidneys, and numerous other organs are often associated with leptospirosis and uremia.<sup>2</sup> Necrotizing endarteritis of the pulmonary artery, aorta, coronaries, and other vessels, sometimes complicated with dystrophic calcification, is commonly observed with uremia due to various causes. Hyalinization of the splenic arterioles is regularly seen in older dogs. Diffuse arterial sclerosis with elastic hyperplasia, rheumatic arteritis, thrombo-angiitis obliterans and endarteritis obliterans are unknown in this animal. A common finding in purulent metritis is a thrombo-arteritis of the pulmonary vessels, and lesions identical with human periarteritis nodosa have been described in dogs.<sup>3</sup>

A survey of the available literature indicates that primary xanthomatosis and secondary xanthomatosis resulting from hyperlipemia have not been noted in any of the tissues of domestic animals although personal observations have demonstrated the presence of foam cells in some neoplasms and inflammatory lesions.

\* Received for publication, July 16, 1945.

## MATERIAL AND METHODS

The reported case was that of a dog brought to my animal hospital for treatment. Euthanasia was performed with soluble pentobarbital given intravenously, followed by immediate necropsy. The tissues were fixed in Zenker's fluid-formalin solution and 10 per cent solution of neutral formaldehyde, U.S.P. Paraffin sections of the former were stained with hematoxylin and eosin, Masson's trichrome stain,<sup>4</sup> Foot's modification of Hortege's silver carbonate method for reticulum,<sup>5</sup> Mallory's phosphotungstic acid hematoxylin, Dominici's stain,<sup>6</sup> Verhoeff's elastica and van Gieson's stains. Formalin-preserved tissue was embedded in gelatin<sup>7</sup> and Lison's<sup>8</sup> tabular method of lipid analysis was applied to frozen sections using sudan IV, Nile blue sulfate, the Lorrain Smith-Dietrich method, and the Schultz and Romieu technics. The von Kossa technic for calcium, and microchemical examination for inorganic and organic iron were performed on frozen sections.<sup>9</sup>

## CLINICAL DATA

The animal was an 8-year-old English setter that had been under my care since 3 months of age. The past history consisted of frequent attacks of gastro-enteritis, the removal of a solitary mast cell tumor (Case 2 of a previous report<sup>10</sup>), and the occurrence of acute interstitial nephritis at the age of 6 years. When 8 years old, the animal was brought to the hospital for anorexia of 6 days' duration, occasional vomiting, weakness, and inability to walk for 3 days. Examination of the urine disclosed a specific gravity of 1.018, a 1 plus albumin test, and occasional hyaline and granular casts. The blood urea nitrogen was 31.0 mg. per cent. A blood count showed 4,880,000 red cells, 8.9 gm. of hemoglobin (Newcomer method), and 22,000 white cells. The differential percentages were: segmented neutrophils, 40; nonsegmented neutrophils, 42; lymphocytes, 5; and monocytes, 13. Bone marrow aspirated from the crest of the ilium by the method previously described<sup>11</sup> indicated: segmented neutrophils, 29.6 per cent; nonsegmented neutrophils, 45.0 per cent; lymphocytes, 6.6 per cent; monoblasts, 0.6 per cent; monocytes, 17.0 per cent; normoblasts, 0.6 per cent; hematogones, 0.2 per cent; and mast cells, 0.8 per cent.

In view of the moribund status of the animal, the owner consented to euthanasia.

## MACROSCOPIC OBSERVATIONS

The gross lesions of the arteries were especially evident in the kidneys, heart, spleen, prostate, liver, lymph nodes, skin, adrenals, and pancreas. In longitudinal and cross sections the arteries were thickened, their walls were prominent and contained numerous, nodular, yellow-tan lesions measuring from 0.1 to 0.2 mm. in diameter (Figs. 1 to 3).

The carotid artery presented a smooth intima with many confluent yellow-tan areas ranging from 0.5 to 2.0 mm. in diameter. The thoracic

and abdominal portions of the aorta contained occasional yellow-tan lesions similar to those in the carotid artery.

The capsules of both kidneys were adherent and the external surfaces nodular. They were uniformly grayish tan and firm to palpation. The striations were obscured.

The auriculoventricular valves were wrinkled and nodular and the semilunar valves were normal. The left ventricle was thickened. Small, irregular, gray areas of fibrosis were seen in the cardiac muscle.

The prostate was approximately twice its normal size. The cut surface was slightly nodular and sections showed lobulation resulting from fibrous trabecular proliferation with numerous small and large cyst-like structures.

The other organs were normal grossly.

### MICROSCOPIC OBSERVATIONS

#### *The Lesions in the Muscular Arteries*

A survey of the different organs revealed similar arterial alterations and, although the atheroma-like process varied in degree and extent in the different vessels, a logical sequence in the development of the pathologic process could be readily established. The vascular involvement was conspicuous in the kidneys, heart, prostate, spleen, liver, skin, lymph nodes, adrenals, and pancreas, with few affected vessels in the remaining organs. The veins were entirely normal. The aorta and carotid artery showed changes similar to those of the muscular arteries (Fig. 8).

As seen in paraffin sections from which fat had been removed, the earliest lesion consisted of groups of foam cells located in the media (Fig. 4). The foam cells varied from 8 to 35  $\mu$  in diameter and were round, oval, or polyhedral. They were closely packed, but a distinct cell membrane clearly outlined each cell. The cytoplasm presented a fine, mesh-like appearance and consisted of delicate strands forming minute round or oval spaces. Many cells contained in addition one or several large cytoplasmic vacuoles. The nuclear structure was vesicular with a few fine chromatin granules. The nuclei were round, oval, irregular, elongated and frequently crenated. Each cell contained usually one, sometimes two, and occasionally three or four nuclei that were centrally or peripherally located. The majority of the nuclei had one or two small central or eccentric nucleoli.

In frozen sections, the lipids in the foam cells existed as small, spherical, closely packed but discrete droplets, and as large, round, oval, or irregular drops and masses (Figs. 10 and 11). Both forms sometimes occurred in the same cell. The fat was largely intracellular,

although the larger droplets and masses were occasionally extracellular. Fine droplets were also present in muscle cells immediately adjacent to the foam cells.

Histochemical studies of the lipids in the foam cells, following the interpretations formulated by Lison,<sup>8</sup> revealed the following data. The lipids were a dirty grayish brown in unstained frozen sections. Treatment with Lugol's solution produced no black-green or brown color indicating the absence of carotinoids; sulfuric acid caused no red color, suggesting the absence of chromolipoids. With the Schultz test the lipids gave a rich blue-green color, and with the Romieu test the initial reddish purple coloration gradually turned green. Both of these modifications of the Liebermann-Burchard reaction are specific for cholesterol and its esters. Mounted unstained frozen sections were doubly refractive under crossed Nicol prisms but showed no cross of polarization (Fig. 9). When the slide was heated, the anisotropic material greatly decreased in amount. With sudan IV the lipids stained a deep red-orange (Fig. 10). The Lorrain Smith-Dietrich method produced a dark blue coloration which is characteristic for a lipin only if cholesterol is absent. Nile blue sulfate produced color ranges from rose to dark blue, with intergrading variations such as lilac, purple, purple-red, and purple-blue occurring in many instances in the same artery (Fig. 11). The rose color signifies the presence of nonsaturated glycerides whereas the blue color is nonspecific.

The collections of lipid-filled cells assumed a nodular form and the larger groups increased the width of the media from two to three times (Fig. 4). They were located either in the external or middle media, and, when in the former, the adventitia was sometimes thinned. The xanthoma-like masses contained only rare argyrophilic and thin connective tissue fibers; elastic fibrils and muscle cells had disappeared. At the periphery of the foam cell conglomerates were seen muscle cells with minute fat droplets in their cytoplasm. These muscle cells showed the normal type of elongated muscle nucleus with few fine chromatin granules and a small nucleolus. Other muscle cells with increased lipid content contained a plumper, more rounded nucleus similar to that of the foam cells lying near them. There were many cells of intermediate form so that numerous transitional stages between normal muscle cells without lipid and the foam cells were present. Usually a normal zone of media existed between the xanthomatous lesion and the intact internal elastica. The adjacent media and the intima underlying the foam cells were normal. This early stage was focal, evinced no inflammatory reactions or fibrosis, and was confined to the media.

In addition to the small focal xanthomatous formations that occurred principally in the larger arteries, numerous smaller arteries showed either diffuse eccentric or concentric replacement of their media with collections of foam cells, so that the width of the media was increased from five to fifteen times (Fig. 5). In these vessels, the inner elastica was usually intact and the intima and adventitia were normal.

Frequently the medial lipidosis extended up to and included the intima (Fig. 6). The internal elastica usually abruptly disappeared at this point and intimal atheromas, somewhat similar to those in man and those experimentally produced in rabbits by cholesterol feeding, sometimes appeared. No intimal atheromas were found, however, without previous involvement of the media. In occasional arteries, small collections of red cells were interspersed between the foam cells.

The next stage observed in the development of the medial lesion was the proliferation of fine connective tissue fibrils and strands in the areas of fatty deposit. These fibrils gradually became coarser and denser (Figs. 4, 6, and 7). The fibrosis was irregular and cellular in the early stages. The connective tissue proliferation partially, but only rarely completely, replaced the foam cells. Frequently argyrophilic fibers and dense fibrous strands encircled individual foam cells. Inflammatory and giant cells were absent, and in the scarred areas muscle and elastic tissue had largely disappeared. The fibrosis was usually associated with arteriolar proliferation even in areas with minimal connective tissue formation.

Occurring simultaneously with the fibrous proliferation, and to a lesser degree in arteries without fibrosis, patchy areas were found infiltrated with material giving the microchemical reactions of iron (Fig. 12). At first, these masses, that stained deep blue with hematoxylin, were thought to be calcium, but von Kossa's stain was negative. In unstained frozen sections the iron deposits were a pale lemon-yellow that contrasted with the yellow-brown of hemosiderin in the spleen. Treatment with potassium ferrocyanide and potassium ferricyanide produced no coloration, indicating the absence of ferric and ferrous salts of inorganic iron. A positive Prussian blue reaction followed exposure to hydrochloric acid and potassium ferrocyanide, proving the presence of organic iron. The iron-containing material existed as small and large round granules, irregular large clumps and masses, and occasionally as crystalline, sheet-like plates. The iron was usually extracellular although small spherules occurred in some foam cells and adventitial histiocytes. Considerable quantitative variation



was found in the different arteries, the iron being absent in some and almost completely filling others.

The accumulative deposit of lipids, connective tissue, and iron was progressive so that the different arteries showed various stages of the pathologic process, transitions often being observed in the same artery. Eventually many arteries showed an irregular concentric distribution affecting all coats, with thickening of the adventitia, and fibrosis and lipidosis extending throughout the media and intima with only occasional strands of the inner elastica persisting (Fig. 7). The vessel lumina were narrowed and completely obliterated in some instances and occasionally filled with foam cells. The pathologic process, therefore, eventually completely transformed the entire structure of the artery (metallaxis).

### *The Lesions in the Parenchymal Organs*

As has been previously stated, the arterial lesions were similar in all organs examined. In addition, various organic alterations were observed, commonly encountered in older dogs and unrelated to the vascular changes.

#### *Kidneys*

The renal lesion consisted of subacute interstitial nephritis and was of the usual type seen in dogs.<sup>12</sup> There were areas of connective tissue proliferation with interstitial infiltrations of lymphocytic cells. The glomeruli showed various changes consisting of distortion of the tufts, fibrosis, and periglomerular fibrosis, but many were normal. Numerous collecting tubules in the medulla were greatly dilated and hyperplastic. Hyperplastic proximal convolutions, in addition to dilated tubules with flattened epithelium, occurred in the cortex.

Besides the arterial lesions described above, lipid stains revealed that the lumina of the interstitial capillaries and of many arteries and veins contained an amorphous material that was pale yellow-orange with sudan IV, pale purple with Nile blue sulfate, pale blue-black with the Lorrain Smith-Dietrich method, gave a positive Liebermann-Burchard reaction, and was doubly refractive with the polarizing microscope. Similar material occurred in the glomerular capillaries and in some Bowman's spaces. Minute fat droplets were frequently present in the glomerular tufts. The epithelial cells of many collecting tubules and some hyperplastic collecting tubules contained numerous fat droplets. The cells of the remainder of the nephron occasionally had fat droplets in their cytoplasm. The physiologic fat-rich terminal portion of the proximal convolution, however, showed a striking reduction in the lipid content of the epithelial cells, a finding not unusual in canine interstitial nephritis. In the interstitial tissue a moderate num-

ber of connective tissue cells and macrophages contained fine fat droplets. Iron-staining material in the form of granules and small clumps occurred in some interstitial macrophages.

### Prostate

Large areas of cystadenomatous hyperplasia associated in some regions with fibrosis and round-cell infiltrations alternated with relatively normal areas of prostatic tissue. These changes are commonly seen in old dogs.

With lipid stains the lumina of many cystically dilated acini and of a few normal acini contained an amorphous material similar in staining reactions to the amorphous material in the vascular lumina. In addition, numerous small and large fat droplets were dispersed throughout this material that gave identical but deeper staining reactions. Fat droplets staining like the arterial lipids occurred in some epithelial cells of the glandular tissue, macrophages, fibroblasts, and muscle cells. A few iron granules occurred in occasional fat-filled histiocytes and in the amorphous lipid-staining material of the glandular lumina.

### Spleen

The general splenic structure was maintained and the changes were those usually seen in older dogs. There were increased numbers of megakaryocytes, a moderate amount of hemosiderin that was principally intracellular, a mild degree of atrophy, focal congestion of some sinuses, and few lymph nodules. The central arterioles showed the hyalinization that is characteristic in dogs over 5 years of age.

In paraffin sections foam cells were absent in the hyalinized arterioles but fat stains indicated ample quantities of small and large lipid droplets, that stained as did the lipids in the muscular arteries. Fat droplets were present in some histiocytes, often associated with hemosiderin. The vessel lumina contained material similar to that seen in other organs but amorphous lipid-staining material rarely occurred in the venous sinuses. The arteries within the spleen were involved with siderosis more frequently than those of other organs. The fatty hyalinized arterioles, on the other hand, rarely showed iron deposition.

### Liver

The liver was normal with the exception of congestion of some sinusoids, hemosiderosis of many Kupffer cells, and occasional vacuolization of hepatic cells.

In addition to the lipid amorphous material in the vascular lumina, similar material occurred in some sinusoids and perivascular lymph

phatics. The bile duct epithelium contained many lipid droplets. Discrete fat droplets of the physiologic type were present in the hepatic cells. These stained orange-red with sudan IV, blue with Nile blue sulfate, blue-black with the Lorrain Smith-Dietrich method, and were negative to the Liebermann-Burchard reaction. The Kupffer cells contained relatively few fat droplets that tinctorially resembled the lipids in the foam cells. Iron-staining material occurred only in the Kupffer cells.

### Other Organs

The remaining organs, with the exception of mild testicular atrophy, pulmonary edema, hypoplastic bone marrow, fibrosed auriculoventricular valves, and focal areas of myocardial fibrosis, were normal. The vascular lumina of all organs, however, contained amorphous lipid-staining material identical with that in the renal vessels.

### COMMENT

The combination of pathologic processes here described, consisting of a deposit of fat in foam cells in the arterial media with a subsequent fibrosis and siderosis that progressively affected all coats of the arteries, can be suitably summarized as athero-fibro-siderosis. It is unnecessary to discuss at length the experimental arterial lesions that have been produced by various means since comparable changes have not been induced. Similar spontaneous vascular lesions are unknown in any other species, including man. This widespread medial lipidosis of the muscular arteries therefore is unusual, for fat deposition in human pathology is much less frequent in muscular than in elastic arteries and medial fat is rare except as an extension of atheromatous foci in the intima.<sup>13</sup> In the rare arterial involvement of human xanthomatosis, the intima is also the site of lipidosis.

The arterial lesions can be interpreted either as a peculiar and unusual type of atherosclerosis with medial localization of the fatty deposits or as a vascular manifestation of that disturbance of lipid metabolism known as xanthomatosis. The latter concept appears more plausible in view of the histologic appearance of the lesion. Although the general histologic picture of the canine vascular involvement resembled the xanthomatous lesions in man, certain differences existed. In the latter, in addition to foam cells and fibrosis, inflammatory cells, Touton giant cells, plasma cells, and eosinophils may be present. These cellular elements were all conspicuously absent in the dog.

Thannhauser<sup>14</sup> has classified the xanthomatous diseases into primary essential xanthomatosis, secondary xanthomatosis due to hyperlipemia, and localized xanthoma formation in inflammatory tissue and

true tumors. The secondary xanthomatosis due to hyperlipemia occurs in diabetes mellitus, chronic pancreatitis, glycogen storage disease, and lipid nephrosis. These conditions were absent in this animal, and, furthermore, the microscopic lesions of human secondary xanthomatosis resulting from hyperlipemia differ considerably from the arterial changes here described. Beside the fact that the arteries alone were affected in this case, in human hyperlipemia foam cells are sparse and extracellular fat deposits occur in the inflammatory connective tissue. That the arterial lipidosis exemplified local xanthoma formation in inflammatory tissue can be discounted from the histologic appearances since the deposit preceded the tissue reaction. The evidence suggests, therefore, that the arterial lesions can be classified with those belonging to the group of primary essential xanthomatoses. In the latter the blood cholesterol may be either normal or elevated, and it is of interest that in man, as in this case, the vascular lesions are usually associated with hypercholesteremia.

Different theories exist as to the mechanism of xanthoma formation. Pick and Pinkus<sup>15</sup> suggested that the hypercholesteremia led to an increase of this substance as well as other fats in the cells. Aschoff<sup>16</sup> believed that the reticulo-endothelial system took up and retained the lipids from the blood stream. Bloch,<sup>17</sup> Schaaf,<sup>18</sup> and Schaaf and Werner<sup>19</sup> considered an extracellular general metabolic disturbance of lipids at fault and that lipids are secondarily deposited in the reticular cells and tissue. Thannhauser and Magendantz<sup>20</sup> distinguished etiologically between essential and secondary xanthomatosis. They considered essential xanthomatosis a systemic disorder of the intracellular metabolism, and secondary xanthomatosis the result of cholesterol infiltration and deposition from hyperlipemic serum.

The relationship of the hypercholesteremia to the vascular lesions in this case requires further comment. It is well known that, contrary to results in rabbits, it is difficult to induce hypercholesteremia in dogs. However, elevation of the blood cholesterol occurs normally in carnivores during pregnancy and following castration.<sup>21</sup> In addition, persistent hypercholesteremia has been experimentally produced in these animals but atheromatosis has never been observed.<sup>21</sup> Cholesterol feeding to dogs results in the deposition of neutral fats and cholesterol in hepatic cells,<sup>22</sup> while in this instance the liver fat gave a negative Liebermann-Burchard reaction. In spontaneous canine diabetes mellitus<sup>23</sup> and obstructive jaundice,<sup>24</sup> the blood cholesterol is increased but atheromatous vascular lesions are absent. This evidence therefore casts doubt on the assumption that the hypercholesteremia which was present in this case led to the arterial lipidosis.

Different opinions have been expressed concerning the derivation of the foam cells. Their origin has been summarized by Gruenfeld and Seelig.<sup>25</sup> Spindle cells of hypertrophied connective tissue, fibroblasts, endothelial cells, degeneration of muscle cells in xanthoma of the eyelids, Kupffer cells, reticulum and periadventitial cells, cells of pulmonary alveoli, glandular epithelium, histiocytes, nerve cells, and the cells of numerous tumors have been considered. Differentiation, of course, must be made between primary and secondary xanthomatosis with respect to the progenitors of the foam cells.

The histogenesis of the arterial lesions in this instance can be traced to the early xanthoma-like masses in which transitional forms between normal muscle cells and foam cells were observed. The concept of muscle transformation into lipid cells gains support from the fact that the media of muscular arteries consists almost exclusively of smooth muscle cells with thin reticular fiber membranes and thin elastic networks.<sup>26</sup> In addition, transitional stages indicating the formation of foam cells from the occasional subendothelial macrophages and adventitial histiocytes were not seen. The endothelial cells can be eliminated, since in the early stages fat droplets were absent from them and the process was deep in the media. Nor is it likely that the rare fibroblasts of the subendothelial region or media took part in the formation of foam cells because these cells appeared in large numbers only later in the process as part of the tissue reaction and did not contain fat. The possibility of mononuclear cells derived from the blood appearing as small lymphocytic and monocytic cells and serving as the parent cell of the xanthomatous tissue, as suggested by Anitschkow<sup>27</sup> in experimental atherosclerosis in the rabbit, is negated by the absence of similar cells in the described lesions.

A consideration of the nature of the fats seen in the foam cells and medial deposits becomes involved in the difficulties inherent in the interpretation of fat stains for the identification of lipids. These difficulties have been demonstrated by Arndt,<sup>28</sup> Kaufmann and Lehmann,<sup>29</sup> Lison,<sup>8</sup> and others. My histochemical studies followed the interpretations of Lison,<sup>8</sup> and indicated that the arterial fats in this case consist of nonsaturated glycerides and cholesterol or its esters. In man the lipids in xanthomas and atheromatous deposits are also mixtures of fatty substances<sup>14,30,31</sup> while in the canine nodular intimal aortic plaques the fat, when present, is neutral.<sup>3</sup>

Collection of iron-containing material is also an unusual lesion in arteries. The siderosis of the vascular walls of the globus pallidus and the brown, iron-staining pigment in the thickened intima of small and medium-sized arteries in pulmonary sidero-silicosis in man are dif-

ferent from the lesion in this dog in their pathogenesis and histology. In human arteriosclerosis, iron is often found in association with calcification<sup>30, 32, 33</sup> but no mention is made in the literature of the presence of iron alone in arteriosclerosis or in any experimental vascular lesion. In human xanthomatous lesions, hemosiderin and red cells are not uncommon. Iron pigment and erythrocytes may also occur in Gaucher cells but are usually absent in Niemann-Pick cells.<sup>34</sup> The iron-containing material in this instance was apparently not identical with hemosiderin, as the latter did not stain with hematoxylin but remained a golden yellow while the iron-containing material stained deep blue. The source of the iron-staining material is problematical in the absence of sufficient hemorrhage with consequent disintegration of red cells in the xanthoma masses to explain the widespread siderosis.

### SUMMARY

An 8-year-old dog showed at necropsy vascular lesions that have not been described as occurring spontaneously or following experimental procedures. These can be classified as a primary essential xanthomatosis of the hypercholesteremic type. Initially, foam cells containing nonsaturated glycerides and cholesterol or its esters appeared in the media of muscular arteries and to a lesser degree in the elastic arteries. The histogenetic evidence indicates that the lipid-containing cells were derived from the smooth muscle cells of the media. Subsequently, connective tissue proliferation developed in which arteriolar formation was prominent. Iron-staining material that differed tinctorially from hemosiderin occurred in the majority of arteries involved in the xanthomatosis. Eventually, in many vessels the entire structure was transformed (metallaxis) by the combination of atheromatosis, fibrosis, and siderosis.

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[ *Illustrations follow* ]



## DESCRIPTION OF PLATES

### PLATE 109

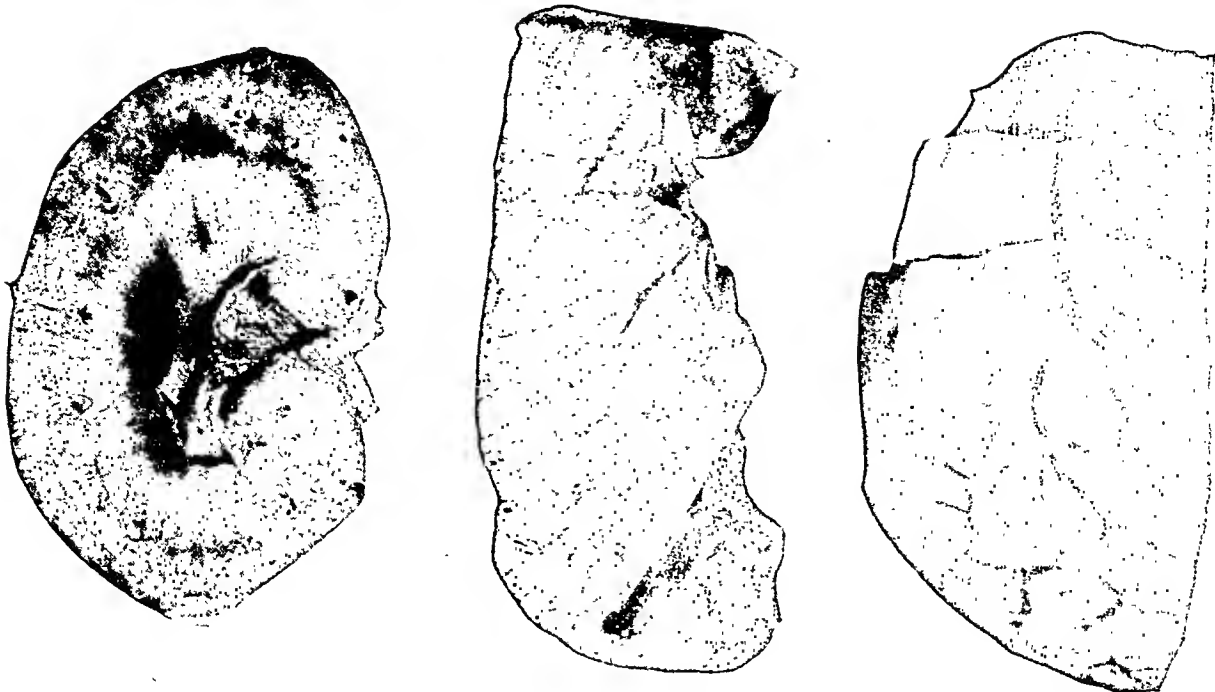
FIG. 1. Section of kidney showing the prominent thickened arteries in the cortex. Approximately  $\times 34$ .

FIG. 2. Section of hypertrophied left ventricle with prominent arteries. Approximately  $\times 34$ .

FIG. 3. External surface of portion of heart muscle showing the thickened coronaries, the walls of which contained small nodular lesions. Approximately  $\times 34$ .

FIG. 4. Longitudinal section of renal artery showing an early xanthoma nodule in the upper left segment of the vessel. The foam cells extend up to, but do not involve the adventitia. The internal elastica is normal and the adjacent media evidences no changes. The lower right segment of the same artery illustrates more advanced changes in the lesion. The inner elastica has disappeared and foam cells can be seen in the intimal region. The xanthoma mass has increased in size and there is partial replacement with fibrous connective tissue, although many foam cells are encircled by connective tissue fibers. Verhoeff's elastica and van Gieson's stains.  $\times 100$ .

FIG. 5. Cross section of a smaller renal artery than that shown in Figure 4 with eccentric thickening of the media by foam cells. The internal elastica is normal and foam cells are absent in the intima. This is an early stage in the lesion and fibrosis is absent. Verhoeff's elastica and van Gieson's stains.  $\times 120$ .



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Bloom

Xanthomatosis of the Arterial Media

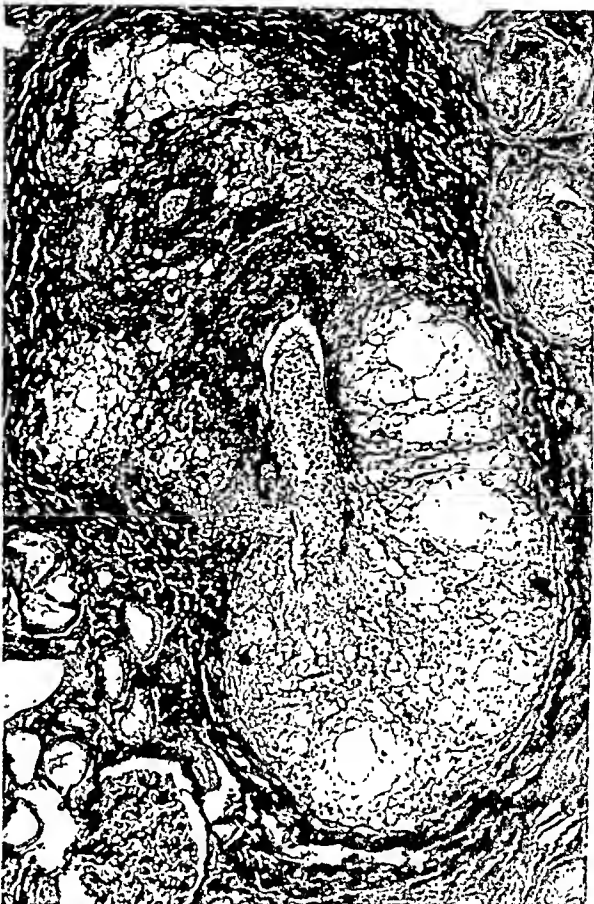
## PLATE 110

- FIG. 6. Cross section of a small renal artery demonstrating a later developmental stage of the medial lesion. Above, a small segment of the artery is normal and, although the inner elastica persists in several areas, the media shows replacement with foam cells with a moderate proliferation of connective tissue. In the lower left segment, foam cells are present in the thickened adventitia. The lumen contains foam cells. Verhoeff's elastica and van Gieson's stains.  $\times 150$ .
- FIG. 7. Cross section of a renal artery with foam cells, connective tissue, and iron-staining material. The media is greatly thickened and the upper portion of the vessel shows fibrosis in which there are arterioles alternating with foam cells. The dark-staining granules in the media are iron-containing material. The elastica has disappeared and the normal arterial structure is completely transformed. Verhoeff's elastica and van Gieson's stains.  $\times 100$ .
- FIG. 8. Cross section of portion of carotid artery. Above, the artery is normal. Immediately adjacent there is foam-cell formation in the media with disappearance of many elastic fibers and moderate fibrosis. Verhoeff's elastica and van Gieson's stains.  $\times 65$ .
- FIG. 9. Frozen section of the same renal artery as in Figures 10 to 12, showing the large amount of anisotropic lipids seen with the polarizing microscope.  $\times 150$ .

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Bloom

Xanthomatosis of the Arterial Media

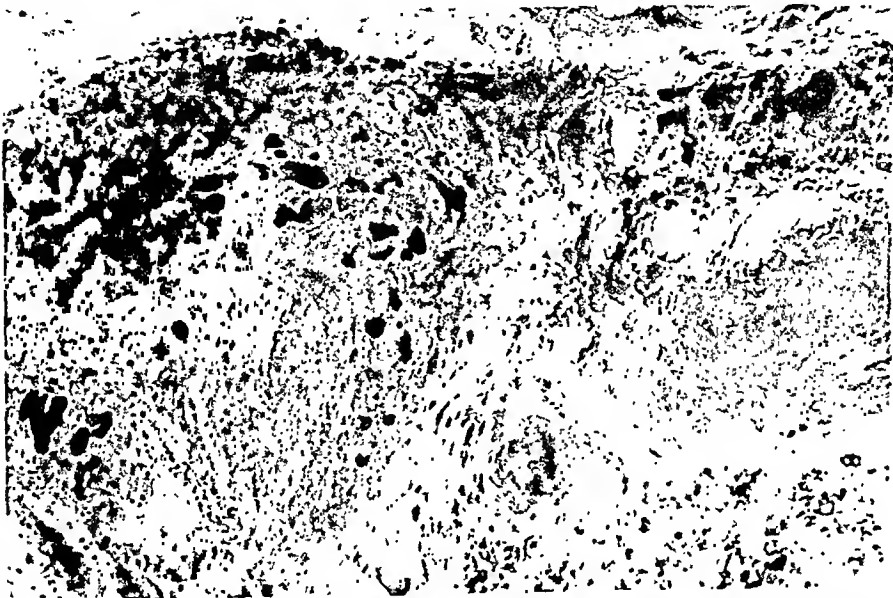
### PLATE III

- FIG. 10. Adjacent section to that shown as Figure 9, illustrating the lipids in the foam cells. The lipid distribution is identical with that in the preceding figure. The amorphous deep-staining masses in the right portion of the photomicrograph are iron-containing material. Sudan IV and hematoxylin stains.  $\times 150$ .
- FIG. 11. Serial section of the same artery as in Figure 9, stained with Nile blue sulfate. The lipid distribution is identical with that in the two preceding figures. The rose-colored lipids signify the presence of nonsaturated glycerides, whereas the blue-stained fat is nonspecific.  $\times 150$ .
- FIG. 12. Serial section of the same artery as in Figure 9, demonstrating a positive Prussian blue reaction of the amorphous, deep-staining material in the right portion of Figure 10. Counterstained with carmine.  $\times 150$ .

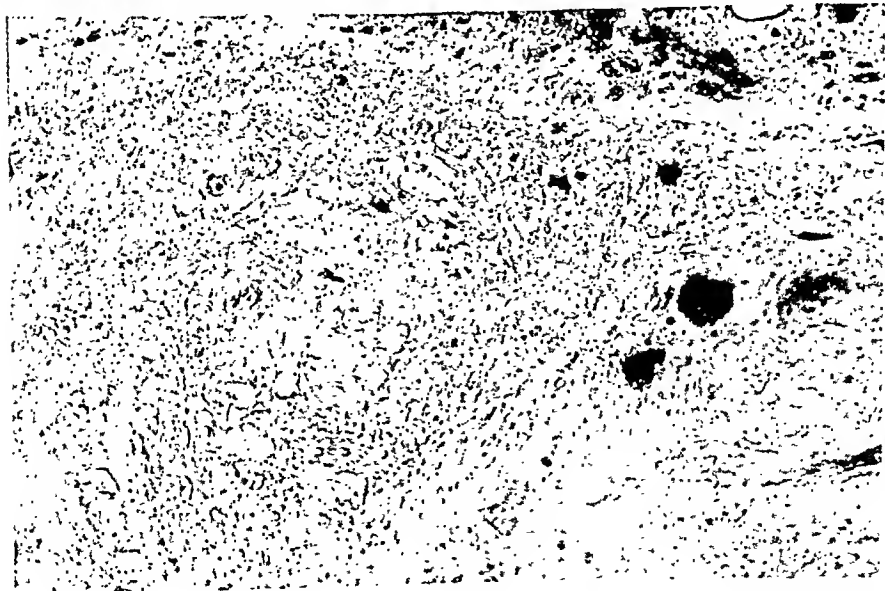
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Bloom

Xanthomatosis of the Arterial Media



## MALIGNANT GRANULOSA CELL TUMOR WITH PSEUDOTUBERCLES \*

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Granulosa cell tumors of the ovary are being reported in increasing numbers. They constituted 2.11 per cent of 1,728 ovarian tumors, according to statistical studies compiled by Szathmáry,<sup>1</sup> Klasten,<sup>2</sup> and Fauvet.<sup>3</sup> Thornton<sup>4</sup> stated that "granulosa cell tumors constitute 8 to 10 per cent of all ovarian carcinomas."

Granulosa cell tumors may be encountered at any age. Holmes and Hauck<sup>5</sup> reported 60 per cent occurring after the menopause, 30 per cent during the child-bearing period, and 10 per cent before puberty. In this connection it is interesting to note the report of a case by Banks<sup>6</sup> in an infant 17 months of age.

The majority of granulosa cell tumors are considered benign, only 5 to 10 per cent being malignant, according to Te Linde.<sup>7</sup> Recent reports of malignant forms are those of Norris,<sup>8</sup> McCartney,<sup>9</sup> Henderson,<sup>10</sup> and Harris.<sup>11</sup> In these reports, except that of Norris, the criteria of malignancy were recurrences or metastases. In Norris' case there were numerous mitotic figures and the tumor was of diffuse type. According to Schiller<sup>12</sup> such histological features, however, are usually not considered as entirely reliable for the demonstration of malignancy in a granulosa cell tumor. The only other criterion of malignancy sometimes given is that stated by Pratt,<sup>13</sup> according to whom these tumors are more often bilateral when malignant. In Klasten's series,<sup>2</sup> 6.2 per cent were bilateral. Dockerty and MacCarty<sup>14</sup> stated that 90 per cent were unilateral.

Conflicting views are expressed as to the histogenesis of granulosa cell tumors. It was formerly accepted that granulosa cells originated from the celomic epithelium. Morehead and Bowman,<sup>15</sup> in a recent article, took issue with this theory of origin and contended that the granulosa cells are derived from the mesenchyme. If this assumption is correct, it would explain the extra-ovarian location and occurrence of granulosa cell tumors of the uterus, broad ligament, and retroperitoneum.

The well-recognized histological types are folliculoid, cylindromatous, and diffuse. The presence of Call-Exner bodies in these tumors is usually considered diagnostic. Another feature which aids in the diagnosis is evidence of estrogenic hormonal activity as reflected by

\* Received for publication, May 29, 1945.



the proliferative "Swiss-cheese" type of endometrium. According to Wolfe and Kaminester,<sup>16</sup> however, secretion by these tumors may be lacking, especially in those which are immature and rapidly growing and show a diffuse or medullary pattern. On the other hand, estrogen production may be so marked as to be considered the carcinogenic agent for a malignant neoplasm of the endometrium by its influence, according to Stohr.<sup>17</sup> Dockerty and MacCarty<sup>18</sup> stressed the low degree of malignancy of granulosa cell tumors and discussed their radiosensitivity. The association of adenocarcinoma of the uterus with a granulosa cell tumor of the ovary was reported. These authors also emphasized the importance of excess theelin as a carcinogenic agent. An increasing number of carcinomas of the endometrium are being reported in connection with granulosa and theca cell tumors of the ovary. Recently reported cases are those of Ingraham, Black, and Rutledge,<sup>19</sup> three cases, and Stohr,<sup>17</sup> three cases. It is doubtful whether adenocarcinoma of the uterus can be explained on the carcinogenic action of theelin alone. The extensive endometrial hyperplasia, with the glandular elements remaining in the "persistent proliferative phase" over a long period of time, resulting from excess estrin production, no doubt plays an important rôle. The importance of suspecting granulosa cell tumor in any patient past the menopause complaining of vaginal bleeding, especially when curettage reveals endometrial hyperplasia, should be stressed.

An unusual and interesting report of a case is that of Murray, Dockerty, and Pemberton,<sup>20</sup> who discussed the coexistence of granulosa cell tumor and ovarian teratoma containing thyroid tissue.

We have recently encountered a case in which there were bilateral tumors of the ovaries with many mitotic figures and well defined Call-Exner bodies. Metastases to the omentum and extension to the fallopian tubes and uterus were also present. In addition, definite pseudotubercles were found histologically in these tumors. Aside from the mention of pseudotubercles by Ewing<sup>21</sup> and the description of giant cells in a metastasis in the brain by McCartney,<sup>9</sup> this feature has not been emphasized in connection with granulosa cell tumors. Because of the peculiar and unusual histopathological structure which might lead to confusion in diagnosis, it was felt that a report was warranted.

#### REPORT OF CASE

The patient was a colored female, 38 years of age, who was admitted to the hospital on January 26, 1942, complaining of a "pressing down feeling" in her abdomen. Her abdomen had been becoming more and more protuberant since April, 1941. During this time there was gradually increasing lower abdominal

discomfort. She was having a scanty vaginal discharge of blood. Her preceding menstrual period was of 3 days' duration during the second week of December, 1941. The flow at that time was scanty. Before December, 1941, menses occurred every 28 days, lasting for 3 or 4 days, with no indication of menorrhagia, or dysmenorrhea. She was gravida 12, and para 9; she had five living children.

On examination, the temperature was 98.6° F.; pulse, 80; and respiration, 20 per minute. The patient was poorly nourished, though well developed. A small subcutaneous nodule was noted in the left hypochondrium. The thyroid gland was symmetrically enlarged; no signs or symptoms of toxicity were manifested, however. The abdomen was markedly prominent and without tenderness or rigidity. A smooth, round, freely movable mass was palpated in the lower abdomen. On vaginal examination, cystocele, rectocele, and chronic cervicitis were noted. The uterus was anterior, but displaced to the left. The left cornu of the uterus was attached to, or continuous with, a large mass posterior and to the left of the uterus, extending downward into the cul-de-sac. This mass was firm and could not be completely displaced out of the pelvis. It was described as being about the size of a uterus with 4½ to 5 months' pregnancy.

On February 5, 1942, a laparotomy was performed. Large tumors of both ovaries were encountered. There was also a yellowish brown, firm nodule, measuring 0.5 cm. in diameter, in the omentum. Bilateral salpingo-oophorectomy, hysterectomy, and excision of the omental mass were performed.

Postoperatively, the patient developed abdominal distention, nausea, and vomiting. Her course was a stormy one, but she recovered satisfactorily and was discharged on the 40th postoperative day.

### *Gross Findings*

The specimens submitted for examination were a portion of omentum, a uterus, two fallopian tubes, and two ovarian tumor masses. The portion of omentum measured 6.5 by 1.5 cm. It was thin and pale gray, with a few small, yellow, granular areas and a larger yellowish brown, firm nodule measuring 0.5 cm. in diameter. The uterus and cervix together measured 10 by 6 by 4 cm. Just above the internal os, the endometrium was slightly roughened and granular. On the postero-superior surface of the uterus there was an elevated area, 2.5 cm. in diameter, which was soft and yellowish brown. It appeared to extend for a short distance into the myometrium. The serosal surface of the right fallopian tube was smooth except distally where it was granular and yellowish brown. The left fallopian tube showed no tumor implantation on its surface. The two tumor masses with portions of the ovaries were separate from the uterus. The larger one measured 18 by 15 by 8 cm. On its superior surface a portion of fallopian tube was present. The external surface of this tumor mass was lobulated (Fig. 1). Numerous areas of hemorrhage were noted. The rest of the tumor was yellowish brown. At the lower pole, a thick-walled cavity, measuring 5.5 cm. in diameter, was noted. The inner surface was smooth and pale and an area of necrosis, 2 cm. in diameter, formed part of its boundary. The tumor, in general, was soft and mushy. The cut sur-

face was yellowish brown and appeared nodular. The other tube and ovary made up a small mass measuring 11.5 by 8.5 by 6 cm. This mass was very soft and had a lobulated external surface showing several large cysts varying in size from 2.5 cm. to 4.5 cm. in diameter. The largest of these was filled with a serohemorrhagic fluid. The cysts were thin-walled and transparent. The portion of fallopian tube attached to this mass showed some small nodules on its external surface. The tumor proper was light yellowish brown, nodular, and edematous, with only small areas of necrosis.

### *Microscopical Findings*

On histopathological examination, the ovarian tumors were found to be made up largely of diffusely arranged cells with round, dark nuclei surrounded by very small amounts of cytoplasm. In some areas these cells were quite closely packed, while in others they were widely separated. Here and there, rather well defined follicular structures with ovum-like bodies in their centers (Call-Exner bodies) were seen (Fig. 2). Much degeneration and necrosis were present. Scattered throughout the sections were numerous pseudotubercles made up of epithelioid cells and some giant cells, and occasionally showing small central areas of necrosis (Fig. 3). The giant cells contained many nuclei arranged at the periphery in some and in the center in others (Fig. 4). Lymphoid cells were not seen. The tumor cells showed mitotic figures in moderate numbers. In one area, lutein cells were arranged in a cyst wall. The cyst contained pink-staining, homogeneous material.

Sections of the fallopian tubes showed extensive invasion with tumor cells. In most areas the tubal mucosa was intact, with the neoplasm destroying the muscularis and serosa. Invasion of uterine musculature and endometrium was also noted. In those areas where the endometrium was not invaded it was thickened and contained straight tubular glands compatible with the persistent proliferative phase (Fig. 5). While some of these glands appeared dilated, no well defined cystic structures suggestive of a "Swiss-cheese" pattern were seen.

The omentum also showed collections of tumor cells which were similar in all respects to those in the ovaries, including the presence of Call-Exner bodies.

*Pathological Diagnoses.* (1) Granulosa cell carcinoma of ovaries (bilateral) with seeding of peritoneum and invasion of tubes and uterus; (2) metastatic granulosa cell carcinoma of omentum.

## DISCUSSION

The diagnosis of granulosa cell tumor in this case is based on the finding of numerous typical Call-Exner bodies among otherwise fairly typical granulosa cells arranged in a diffuse pattern. Endocrine activity was indicated by a persistent proliferative phase in the endometrium. It is realized that "granulosa-cell-like" groups have been reported in dysgerminomas. Novak and Gray<sup>22</sup> have called attention to the tumors which Kermauner and Nürnberger<sup>23</sup> called granulosa cell carcinomas associated with lesions suggestive of tuberculosis. Schiller,<sup>24</sup> in subsequent examination of these tumors, considered them to be dysgerminomas. Nevertheless, it is felt that the granulosa cell masses in the present case are sufficiently definite and extensive to warrant the diagnosis of granulosa cell tumor rather than dysgerminoma. Opinion that the tumor in this case is malignant rests on its seeding of fallopian tubes and uterus, the invasion of the uterine musculature, the fact that it is bilateral and, of lesser importance, the appearance histologically of numerous mitotic figures.

The particular feature of interest here is the presence of pseudotubercles in a granulosa cell tumor. As has been stated, Ewing<sup>21</sup> has called attention to this occurrence, and McCartney<sup>9</sup> has described giant cells in a metastasis from a granulosa cell tumor of the ovary. Of the so-called "special" ovarian tumors, however, the one more usually associated with pseudotubercles is the dysgerminoma. This feature has been pointed out by Ewing,<sup>21</sup> Novak and Gray,<sup>22</sup> Schiller,<sup>24</sup> and Sailer.<sup>25</sup> Seegar,<sup>26</sup> however, in a rather complete discussion of dysgerminomas, did not describe it.

Sailer<sup>25</sup> discussed the presence of pseudotubercles in dysgerminomas at some length. According to him, several explanations have been offered for their presence. Much of his discussion may also apply to the case of granulosa cell tumors.

Some authors have found an associated adnexal or intestinal tuberculosis in these cases. Therefore they have considered the collections of epithelioid cells, fibroblasts, and giant cells as tuberculous. The possibility of an associated tuberculosis in these cases is an interesting question. Aside from the histological features (pseudotubercles), one is reminded of the syndrome of fibroma of the ovary with ascites and hydrothorax, described by Meigs.<sup>27</sup> Repeated efforts have failed to produce evidence of tuberculous lesions or tubercle bacilli in cases showing this syndrome. In this connection the case of Vogt<sup>28</sup> is interesting. He reported a granulosa cell tumor of the ovary associated with hemoperitoneum and hemothorax. The serosanguineous fluid

disappeared from the chest and abdomen 15 days after removal of the ovarian tumor and had not recurred 2 years later. Others have not found *tuberculous lesions elsewhere*, nor have they demonstrated tubercle bacilli in the lesions in the ovaries.

The most generally accepted explanation is that these pseudotubercles represent a stromal reaction to disintegrating tumor cells. According to Novak and Gray,<sup>22</sup> on the basis of recent laboratory investigations of tuberculosis suggesting that the characteristic tissue reaction is probably produced by fatty substances of fluid nature, it is not unlikely that similar lipids may result from the degeneration of dysgerminoma cells. Since this change may occur in dysgerminomas, it is plausible that a similar finding should be encountered in granulosa cell tumors which also contain lipids. Greenblatt, Greenhill, and Brown<sup>29</sup> have investigated the lipid content of certain ovarian tumors. They found that in such tumors fat may be encountered due to degenerative processes, or as a product of tissue metabolism (hormone storage). The fat in dysgerminomas is believed to be degenerative as it is more abundant in necrotic areas of the tumors. In granulosa cell tumors, on the other hand, it seems likely that variations in lipid content are linked with endocrine metabolism and hormone storage. It, then, is of a different nature from the degenerative lipids of dysgerminomas. In the tumor herein described there was not an unusual amount of endocrine activity, but much evidence grossly and microscopically of degenerative changes. In the light of the work just mentioned this would suggest that there should be little lipid storage of a hormonal nature, but there may well have been deposits of a degenerative nature, thus resembling the lipids of dysgerminomas. The explanation for pseudotubercles in our case may, therefore, be similar to that offered for the presence of these structures in dysgerminomas.

Föderl,<sup>30</sup> in cases of dysgerminomas, has found pseudotubercles in tumor thrombi lying within the lumina of small blood vessels containing no stromal elements. Under these circumstances a derivation from tumor cells or from transformed endothelial cells must be assumed.

#### SUMMARY

A case of granulosa cell tumor is reported which satisfied some of the criteria for malignancy in neoplasms of this group. It also presented the interesting and previously unemphasized histological finding of pseudotubercles within its structure. It is believed that the best explanation for the presence of pseudotubercles is that they represent a stromal reaction to lipids derived from disintegrating tumor cells.

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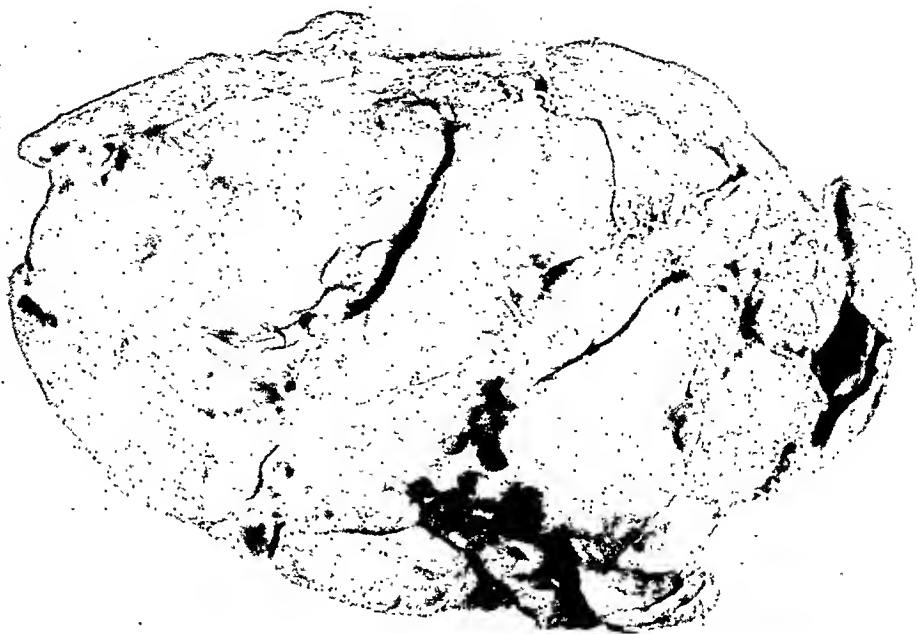
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### DESCRIPTION OF PLATES

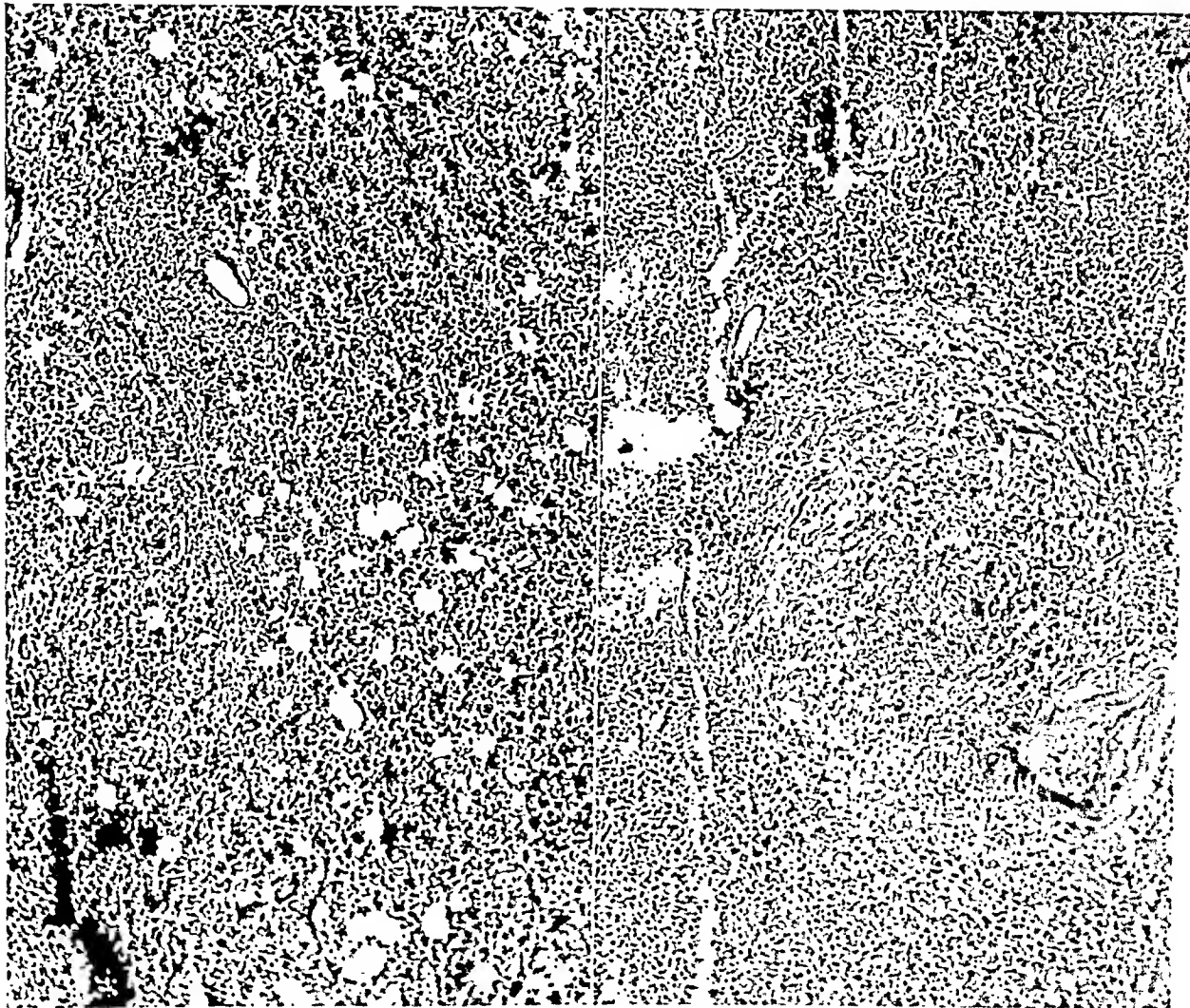
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#### PLATE, II 2

- FIG. 1. Gross picture of one ovarian tumor mass, measuring 18 by 15 by 8 cm. The outer surface was rough and slightly lobulated. The cut section presented a doughy structure with areas of necrosis, some hemorrhage, and a few cystic areas measuring from 1 mm. to 5.5 cm. in diameter.
- FIG. 2. The neoplasm shows numerous Call-Exner bodies, some containing small ovum-like structures. The intervening structure is made up mainly of anaplastic granulosa cells.  $\times 200$ .
- FIG. 3. A pseudotubercle with a small central area of necrosis. The tubercle is surrounded by an even distribution of granulosa cells.  $\times 200$ .



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Schattenberg and Harris

Malignant Granulosa Cell Tumor



PLATE 113

FIG. 4. Two pseudotubercles, one with two giant cells in its periphery. A few Call-Exner bodies are also shown in the surrounding masses of granulosa cells.  $\times 200$ .

FIG. 5. In the endometrium the glandular elements are in the proliferative phase. The endometrium is considerably thickened. A small amount of leukocytic infiltration, mainly lymphocytes, is noted.  $\times 200$ .

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Schattenberg and Harris

Malignant Granulosa Cell Tumor



## DYSGERMINOMA OF THE OVARY \*

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The dysgerminoma † of the ovary is a solid tumor which was formerly thought to be quite rare, although more than 200 cases have been published. This tumor is of considerable interest because of the theories of its histogenesis, its potential malignancy, the enormous size it may attain in a child, and its frequent occurrence in pseudohermaphrodites. The name "disgerminoma," proposed by Meyer<sup>1</sup> in 1931, properly defines the tumor and eliminates confusion in terminology caused by describing it as embryonal carcinoma, seminoma, sarcoma, and under many other names. Barzilai's<sup>2</sup> definition is as follows: "The disgerminoma of the ovary is a germinal tissue tumor made up of cellular elements indistinguishable from the sexually undifferentiated mesenchymal cells of the early gonad."

The tumor is frequently found in childhood. Seégar,<sup>3</sup> in reporting 98 cases, found 44.5 per cent occurring in the ages between 10 and 19 years, with only 10 per cent in women over 40 years. The tumor is bilateral in approximately 28 to 30 per cent of cases, and tumor growth may progress in the remaining ovary subsequent to the surgical removal of one ovary, as occurred in one of my cases (case 1).

The dysgerminoma must be classified as a malignant tumor since it grows rapidly, spreads by implantation, and sets up distant metastases. Kirshbaum and Newman<sup>4</sup> reported the case of a 36-year-old female with metastases to the peritoneum, lungs, both kidneys, and the liver, and it has been estimated that about 25 per cent of cases have extrapelvic metastases at the time of operation.

Underdevelopment of the external or internal genital organs is sometimes associated with dysgerminoma. Novak and Gray<sup>5</sup> found only 3 of their 17 patients to have underdevelopment of the external or internal genital organs, although 4 others exhibited hirsutism, and one had a deep, masculine voice. Long, Ziskind, and Storck,<sup>6</sup> in describing a case of dysgerminoma occurring in a pseudohermaphrodite, pointed out that a large pelvic tumor in a patient with pseudohermaphroditism femininus and infantile genitalia suggests the presence of a dysgerminoma.

\* Received for publication, May 29, 1945.

† The spelling "dysgerminoma" is used in *The American Journal of Pathology*, but in direct quotations the original style will be preserved.

## GROSS DESCRIPTION

Dysgerminomata as found at operation or autopsy vary widely in size from small nodules to enormous tumors weighing more than 5 kg. (case 1). The smaller ones may retain the general shape of the ovary, the larger become spherical or ovoid. The tumor is usually encapsulated, with a smooth surface, although it frequently has a bosselated or knobby surface resembling brain in the gross appearance (Fig. 7). The surface made by cutting is yellow to gray, rather solid and homogenous, with areas of degeneration and hemorrhage in the larger tumors.

## MICROSCOPIC DESCRIPTION

The microscopic picture is usually characteristic: the cells are large and uniform in size, the cytoplasm is granular, the nucleus is large, round, and centrally placed. Basophilic nucleoli are frequently seen. Barzilai<sup>2</sup> stated that the true cellular detail is well seen only in frozen tissue or in celloidin preparations since shrinkage occurs when formalin fixation is used with embedding in paraffin. Thus the cells appear to be much smaller and to have indistinct borders, which has led to controversy regarding the type and has favored a diagnosis of round-cell sarcoma.

The pattern which the tumor cells assume varies from a cord-like arrangement, resembling abortive tubule formation, to a medullary or alveolar form, the tumor cells being separated into small or large clumps by fine connective tissue septa. Lymphocytes are frequently found in this connective tissue, and actual lymph follicles are occasionally seen. Giant cells, resembling the Langhans' cells of tuberculosis, are often found in dysgerminoma. These cells have concentrically-placed, multiple nuclei and are often seen in clumps of lymphocytes, together with epithelioid cells simulating tubercles. The pathogenesis of these giant cells has not been satisfactorily explained although it is generally agreed that there is no relation between them and tuberculosis.

The positive Friedman or Aschheim-Zondek test often found in patients with dysgerminoma is interesting since there is no clinical or laboratory evidence of male or female hormonal stimulation. Spielman and Morton<sup>7</sup> reported a hormonal bio-assay in a case of ovarian dysgerminoma and found a complete absence of estrogenic hormone although prolan A was present but unaccounted for. Some patients with a positive Friedman test have been mistakenly thought to be pregnant but roentgenographic studies usually correct the diagnosis. There is, however, no uniformity, and the comparative rarity of

dysgerminoma as compared with other ovarian tumors makes the test of doubtful diagnostic value.

## REPORT OF CASES

### Case 1

The patient, a white female, 14 years old (no. 11603),\* was first seen by her physician in October, 1939, complaining of enlargement of the abdomen. She had menstruated once at 12 years of age, and again at 13. She had otherwise been in a good state of health. Enlargement of the abdomen had been noted 6 weeks prior to examination, and about the same time her voice became of lower pitch and almost masculine. At that time she was given thyroid extract and theelin for amenorrhea.

Examination was negative except for the abdomen which was enlarged to a size suggesting an 8 months' pregnancy. The mass was in the midline but the borders were indefinite. There was no change in the breasts. The blood pressure was 110/60 mm. Hg; laboratory studies of blood and urine showed no abnormality except that *the Friedman test was positive*.

At operation a solid right ovarian tumor was removed which weighed more than 4 kg. The left ovary was normal in size and appearance.

The tumor was an ovoid mass, measuring 28 by 15 by 12 cm., weighing 4028 gm. The surface was lobulated, yellowish pink and in some areas dark red. There was a pedicle, approximately 8 cm. in length. The surface made by cutting showed multiple nodules made up of soft yellow to yellowish pink tissue, varying in size from 0.5 to 7 cm. in diameter. The tissue between these nodules was firm and fibrous (Fig. 1). The sections showed large cells, arranged in semi-cords, separated by fine connective tissue. Some of these were in a medullary pattern, with giant cells and lymphocytes in the connective tissue stroma (Figs. 2 and 3). The diagnosis was dysgerminoma.

Subsequent roentgenograms of the chest and pelvis showed no evidence of metastases. The patient made an uneventful recovery, and left the hospital on the tenth postoperative day.

The patient was seen at intervals, without complaint, until March, 1943, at which time she was 18 years old. She complained of pain in the right lower quadrant of the abdomen. Her menstrual periods had been normal until 3 or 4 months prior to that time when they occurred every 2 to 4 weeks. Her last menstrual period had commenced 10 days before this examination, and she was menstruating when seen.

Pelvic examination showed a large mass in the left side of the pelvis. The examination was otherwise negative. *The Friedman test was negative*.

A laparotomy was performed, and a solid tumor, arising in the left ovary, was removed. This mass had broken through its capsule and was attached to the parietal peritoneum just below the umbilicus. There was no demonstrable involvement of lymph nodes, and no evidence of distant metastasis.

This second tumor was a solid mass measuring 16 by 10 by 10 cm., and weighing 1779 gm. The surface was nodular, bosselated, yellow to gray, with some dark red nodules. The surface made by cutting

\* Patient of Dr. Jerome Jacobs, Seattle, Wash.

showed soft areas with fibrous septa dividing them. There was evidence of recent hemorrhages. The soft areas cut with a fish-flesh-like consistency (Fig. 4). The sections showed a neoplasm made up of large round and polyhedral cells. They had small nuclei and granular cytoplasm. There were fine and coarse bands of connective tissue dividing these cells into groups. Otherwise they were in a medullary pattern. The diagnosis was dysgerminoma (Fig. 5).

The patient made an uneventful recovery and left the hospital on the eleventh postoperative day. She subsequently received x-ray therapy totalling 8200 r. over four pelvic ports. She has been seen at intervals and there has been no evidence of recurrence or metastasis.

### *Case 2*

The patient was a female, 11 years old (clinic no. 21203),\* who had been seen in a neighboring city on February 3, 1945, with the chief complaint of pain in her abdomen. The child had always been an active healthy schoolgirl whose family had noticed a gradual enlargement of her abdomen for several months. Two days prior to admission to the hospital the child had struck her abdomen against the corner of a desk, following which her abdomen became progressively larger and there was increasing pain. She had never menstruated.

On examination this child showed an enlargement of the abdomen resembling an 8 months' pregnancy. Percussion and palpation showed the enlargement to be due in part to free fluid. Because of the apparent emergency, a Friedman test was not done. Laparotomy was done promptly and a tumor was found occupying the pelvis and lower abdomen. There were 2 to 3 liters of clear straw-colored fluid in the peritoneal cavity, and a large tumor was found arising in the right ovarian region. This was easily removed. There was no evidence of extension or implantation. The left ovary and the remainder of the pelvic genitalia were entirely normal. Recovery was uneventful.

The specimen was a solid, rounded and flattened tumor, measuring 21 by 10 by 10 cm., and weighing 1500 gm. The capsule was smooth; the surface was bosselated and the consistency was soft. It was yellow to gray and red, and the surface made by cutting showed many soft areas which were yellow and cut with the consistency of fish flesh. There were several large pockets containing fluid and clotted blood where the tumor had undergone degeneration. The sections showed a neoplasm with relatively few tumor cells and a preponderance of fibrous connective tissue; the tumor cells were round or polyhedral, and were arranged in small medullary clumps; there were many lymphocytes in the connective tissue and some giant cells (Fig. 6). The diagnosis was dysgerminoma.

### *Case 3*

The patient, a female, 18 years old (clinic no. 114698),† came to the Mason Clinic on January 30, 1945, complaining of soreness in the abdomen of 3 months' duration. The soreness had radiated to the inguinal region on both sides. She

\* Case of Dr. Lawrence Schuler, Port Angeles, Wash.

† Case of Dr. Joel W. Baker, Seattle, Wash.

consulted a physician who prescribed some medicine, but subsequently the patient noticed a slight abdominal swelling unaccompanied by pain.

On February 2, 1945, she fell and immediately afterwards had an acute episode of abdominal pain. She then consulted a doctor who felt that she was pregnant, although a Friedman test was negative. The patient's last menstrual period started on February 2, 1945. Examination showed a large tumor filling the lower abdomen and extending to the umbilicus. Rectal and vaginal examinations showed the cervix to be normal, and a diagnosis of ovarian tumor was made.

Laparotomy was performed on February 9, 1945, and a tumor was found arising in the left ovary and extending from the pelvis into the left flank. The left fallopian tube was long and edematous. The right ovary appeared entirely normal. The tumor was removed without difficulty, and the patient made an uneventful recovery.

The tumor was solid, measuring 21 by 13 by 10 cm., and weighing 1376 gm. The surface resembled the surface of a brain, with deep convolutions, although some of them were flattened. The surface made by cutting showed many nodules which were soft and cut with fish-flesh-like consistency. The tissue between these nodules was firm and fibrous (Fig. 7). Sections showed the tumor cells to be pleomorphic. They had a granular cytoplasm, and there were many mitotic figures. The tumor cells were separated by fine fibrous connective tissue. Many lymphocytes were present, some of them in large clumps resembling a lymph follicle (Fig. 8). The diagnosis was dysgerminoma.

#### SUMMARY

Four instances of dysgerminoma of the ovary are reported, arising in three females. The bilateral tumors of case 1 were not simultaneous. The left ovary appeared entirely normal at the time of the removal of an enormous tumor arising in the right ovary. The second tumor was removed nearly 4 years later.

A Friedman test was done in three cases; it was positive in 1, negative in 2.

In case 2 the tumor was accompanied by considerable ascites which was of recent origin since the abdomen enlarged rapidly following an accident. The presence of ascites has been commented on in previous reports.

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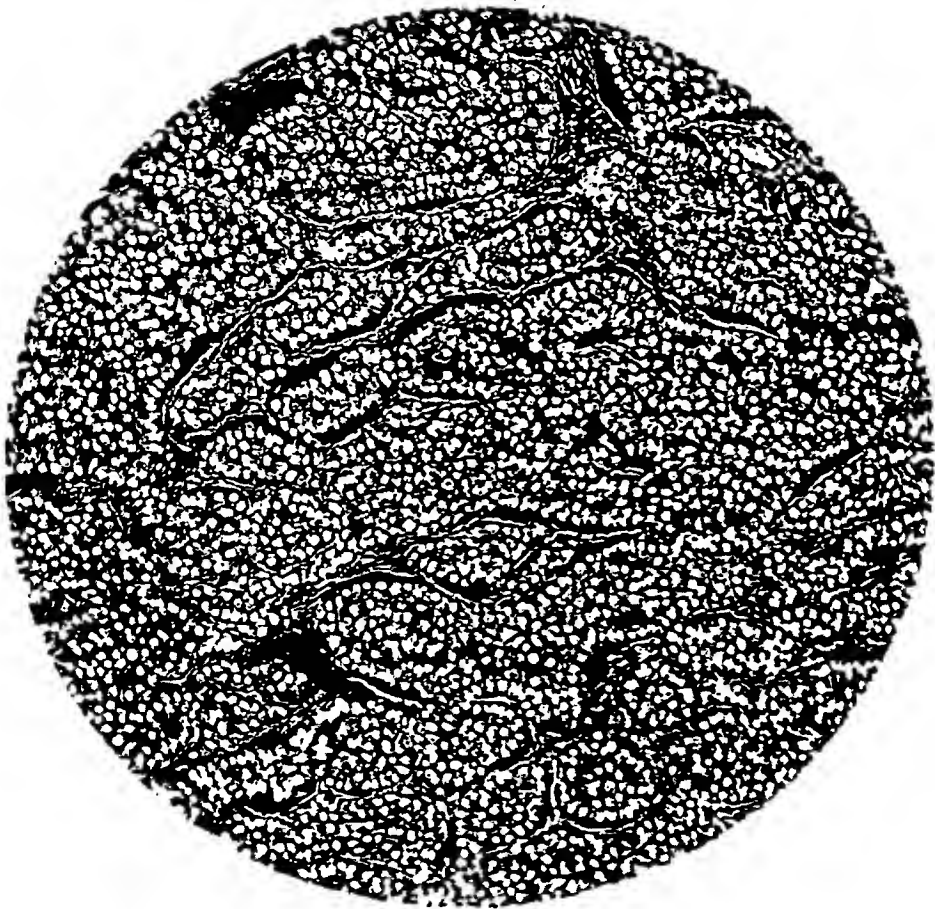
## DESCRIPTION OF PLATES

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### PLATE 114

FIG. 1. Case 1. A cut section of the dysgerminoma, weighing 4028 gm.

FIG. 2. Case 1. Photomicrograph showing a typical field, demonstrating a cord-like or semitubular arrangement. Hematoxylin and eosin stain.  $\times 115$ .



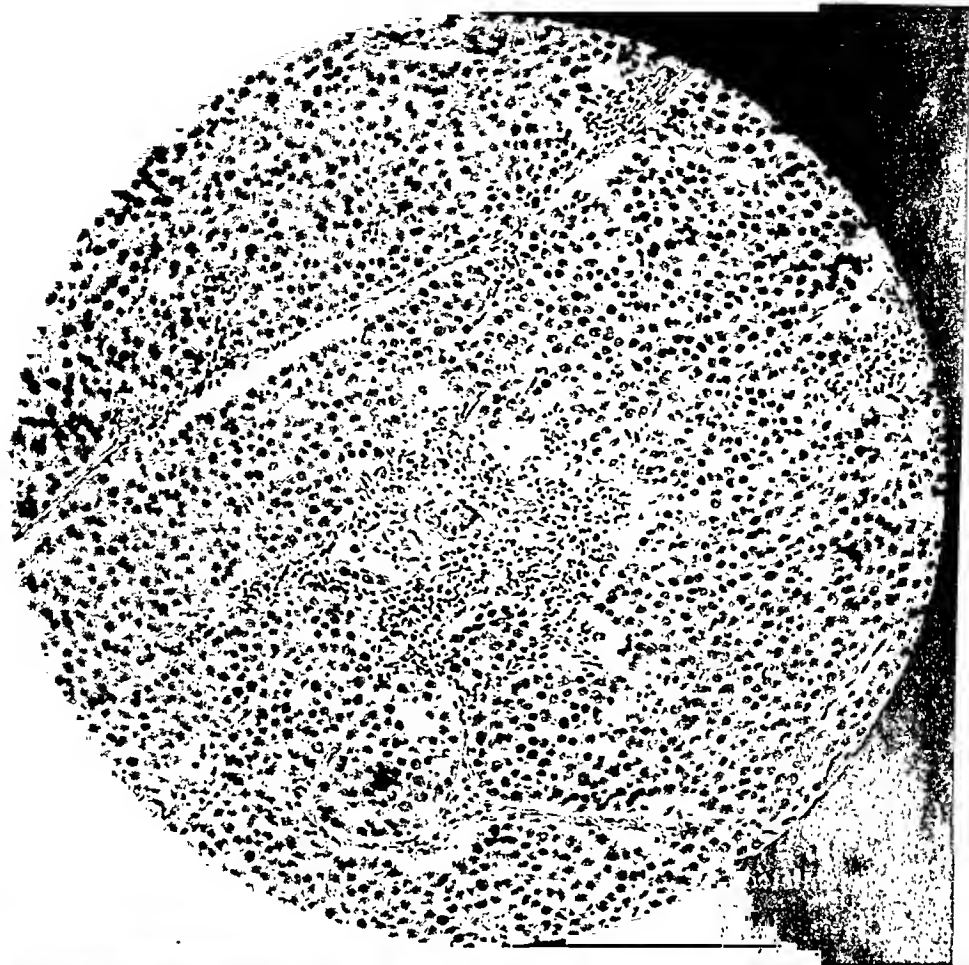
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PLATE 115

FIG. 3. Case 1. Photomicrograph showing a giant cell surrounded by lymphocytes.  
Hematoxylin and eosin stain.  $\times 116$ .

FIG. 4. The gross appearance of the second ovary in case 1.

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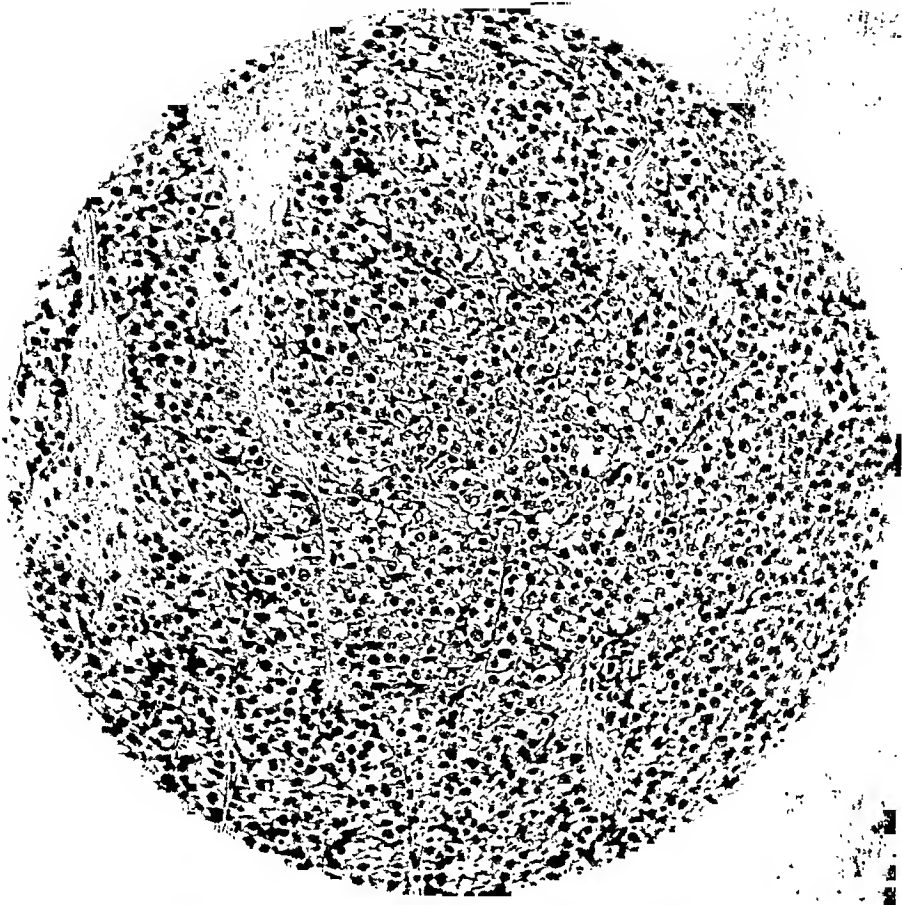
Dysgerminoma of the Ovary

PLATE 116

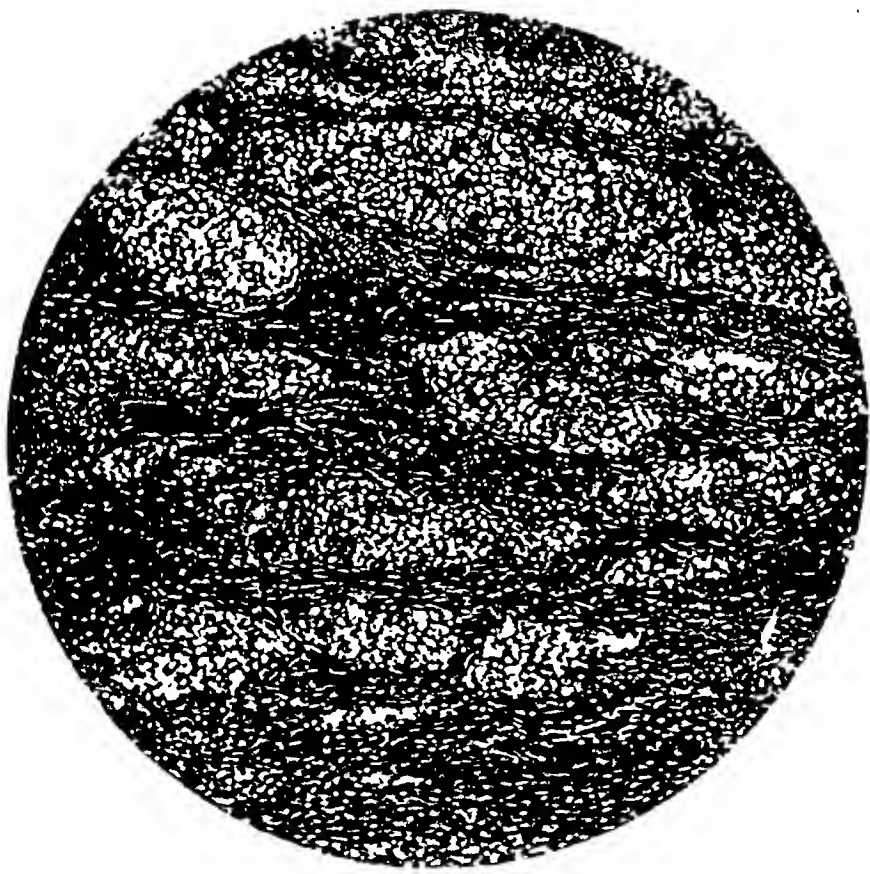
FIG. 5. Case 1. Photomicrograph of tumor shown in Figure 4, showing a more undifferentiated tumor than in Figure 2. Hematoxylin and eosin stain.  $\times 107$ .

FIG. 6. Case 2. Photomicrograph showing a predominance of fibrous connective tissue with many lymphocytes in the stroma. Hematoxylin and eosin stain.  $\times 107$ .

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Dysgerminoma of the Ovary

PLATE 117

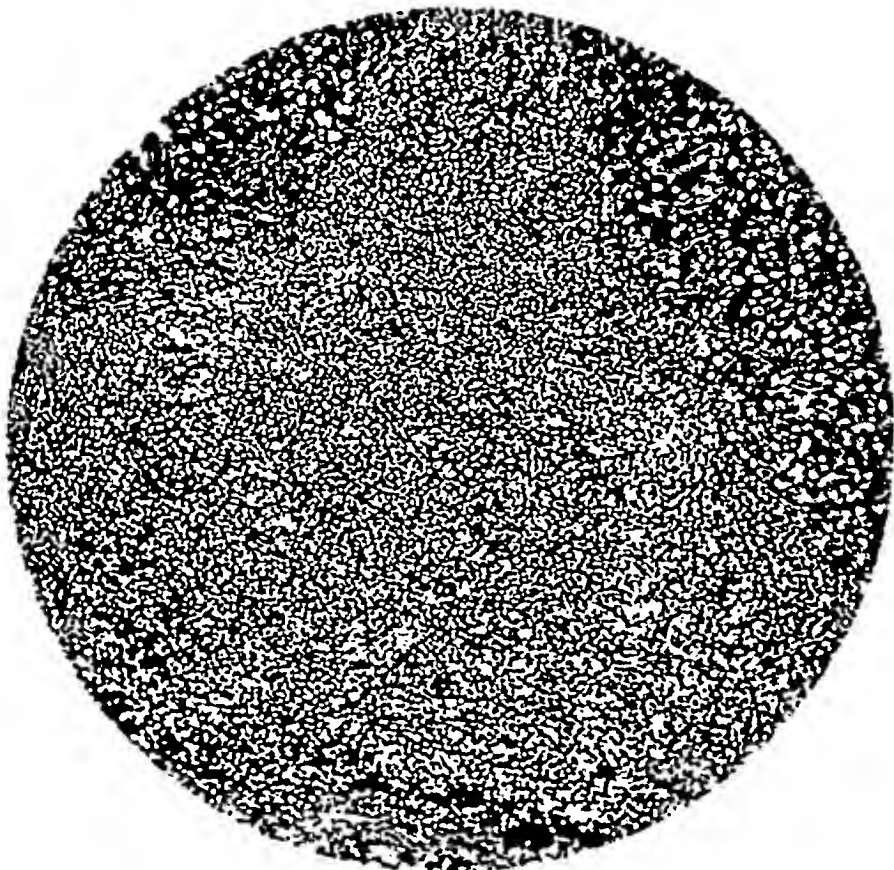
FIG. 7. Case 3. Gross appearance of the tumor, showing a similarity to the surface of a brain.

FIG. 8. Case 3. Photomicrograph, centering over a clump of lymphocytes with tumor cells at the periphery. Hematoxylin and eosin stain.  $\times 110$ .

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# OBLITERATIVE CEREBRAL ARTERIOSCLEROSIS A CHARACTERISTIC VASCULAR SYNDROME \*

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Cerebral arteriosclerosis is the most common and one of the least understood pathologic conditions of the brain. There is no direct relationship between the severity of clinical symptomatology and the degree of sclerosis of arteries in the brain. There are many cases in which, in spite of a far advanced sclerosis of the larger arteries, clinical manifestations are insignificant. On the other hand, in cases with obvious clinical symptomatology considered to be characteristic of cerebral arteriosclerosis, the gross pathologic findings may be minimal or absent.

Possibly the reason for this apparent discrepancy of clinical and anatomical findings has been that most attention was devoted to alterations of the larger arteries; interest has been focused on the gross lesion of arteriosclerotic plaque formation. While this common form of arteriosclerosis has been relatively well studied, little attention has been devoted to the histologic changes of the smaller blood vessels in cases of cerebral arteriosclerosis. These lesions require further detailed study.

In an earlier investigation of vascular changes in 25 cases of hypertensive brain disease an attempt was made to describe a special form of arteriolar alteration typical of hypertension.<sup>1</sup> The view was expressed that these lesions of the arterioles should be separated from the total group of arteriosclerotic changes under the heading of "hypertensive hyaline arteriolopathy."

This presentation will describe a characteristic vascular change frequently observed in cases with clinical manifestations of cerebral arteriosclerosis. This abnormality may be found in combination with sclerosis of larger arteries, or independent of it.

## MATERIAL AND METHODS

This report is based on a detailed study of the cerebral vascular changes in 10 cases of arteriosclerosis. Sections from numerous areas of the brain were stained by the hematoxylin and eosin, cresyl violet, Loyez myelin sheath and Bodian protargol methods, and in some instances with scarlet red stain for fat.

The vascular lesions which are to be described as "obliterative

\* Received for publication, May 29, 1945.

cerebral arteriosclerosis" were encountered in older patients, the ages varying between 68 and 94 years. Both sexes were equally represented. Mental disturbances characterized as those of senile dementia were noted in 5 cases of this series (50 per cent of all cases). Signs of disseminated cerebral lesions (a multiplicity of neurologic signs) were present in 4 instances. In only one case was arterial hypertension (220/120 mm. Hg) noted. In 4 cases there were clinical symptoms of arteriolonephrosclerosis. Evidences of vascular lesions in the peripheral circulation also were found, *i.e.*, in 3 cases there was gangrene of a big toe.

#### PATHOLOGIC OBSERVATIONS

*Gross Findings.* The gross findings in the brain were quite uniform in character. The leptomeninges were often thickened, opaque, and slightly adherent to the underlying cortex. The superior surface of both hemispheres disclosed a moderate degree of convolutional irregularity caused by the presence of numerous minute foci of softening or glial scar formation. Areas of normal tissue frequently alternated with small areas of scarring or with small foci of cystic softening (Fig. 2), producing a slightly granulated appearance of the cortical surface (Fig. 1). The small glial scars often appeared on the surface as tiny depressions. The large vessels at the base were in some cases normal. In others they appeared enlarged, irregular, and tortuous; their walls presented extensive atheromatous change and contained numerous arteriosclerotic plaques. The smaller cortical vessels appeared slightly thickened and tortuous. The larger cisternal as well as the subarachnoid spaces appeared considerably widened.

Coronal sections of both hemispheres revealed in all instances strip-like areas of softening of the cortical ribbon, frequently associated with diffuse atrophy of the cortical gray matter. In some portions of the latter, small areas of softening were so numerous as to give the cortex a mottled appearance. The white matter was grossly normal except for occasional small areas of cystic softening or glial scars varying considerably in size, number, and location. The ventricles were enlarged in all instances.

*Microscopic Findings.* In all cases the leptomeninges were moderately thickened. The subarachnoidal space was distended and harbored small accumulations of fat granule cells. In 4 cases there was dense focal infiltration of the leptomeninges with gitter cells; adjacent portions of the meninges revealed no infiltration. The gitter cell accumulation in the leptomeninges was secondary, as a rule, to numerous small areas of softening localized in the upper layers of the cortical ribbon and communicating with the subarachnoidal space.

The cerebral cortex showed vascular lesions and changes in the parenchyma. The main histologic alterations of the vascular system are illustrated by Figures 3 to 7. The majority of the small arteries exhibited a conspicuous thickening of the intima. The latter showed two principal types of alteration: proliferative and necrobiotic. In the early proliferative stage, as illustrated in Figure 3, the changes were characterized by a cellular proliferation chiefly confined to the inner layer of the vessel. The subendothelial portion of the intima was thickened and very cellular, mainly because of the presence of granulation tissue rich in fibroblasts and mononuclear elements. The tremendous expansion and hyperplasia of the ground substance of the subendothelial connective tissue led to considerable narrowing of the vessel lumen. It was by no means rare that the lumen of the blood vessels was reduced to a mere slit. Cross sections of some of the blood vessels revealed the misshapen and considerably reduced vessel lumen bounded on the outer side by a relatively well preserved media and adventitia (Fig. 4). The proliferative changes, as a rule, were confined to the inner coat, bulging the intima outward and encroaching greatly upon the lumen. The lining endothelial cells showed mild proliferative and no degenerative changes. In addition there was a considerable increase and splitting of the inner elastic membrane in the form of concentrically arranged elastic-laminae. Degenerative changes such as hyalinization of the intima or atheromatous plaque formation were not observed.

In more advanced stages (Figs. 5 to 7) the cells of the subendothelial part of the intima had undergone complete degeneration and necrosis and were replaced by a thin network of connective fibrils harboring a very few fibroblasts and macrophages. These alterations were occasionally associated with secondary atrophy of the media. They were very rarely complicated by fatty or mucoid degeneration. Sclerotic plaque formation or calcification was not observed. As a rule the adventitia was of normal appearance.

Changes of the nervous tissue secondary to the vascular alterations consisted of circumscribed focal areas of softening characterized by the presence of large numbers of fat granule cells (Fig. 2); in addition there were numerous areas of glial scar formation. These focal lesions resulting from insufficient blood supply were disseminated throughout the cortical ribbon (Fig. 1). Only occasionally were they found in the white matter and basal ganglia. In the upper layers of the cortex there were numerous stripe-like devastated areas in which all nerve cells had been destroyed and replaced by glial scar formation. All changes of the nervous tissue were associated with extreme narrowing

or complete occlusion of the vessel lumina. Their variance in severity might be explained by variation in rapidity and completeness of the circulatory disturbance, and by the original caliber of the damaged blood vessel.

The examination of sections taken from other viscera (kidney, spleen, pancreas, liver, lungs, and heart) revealed a moderate degree of arteriosclerosis. Vascular alterations similar to those described as "obliterative cerebral arteriosclerosis" were not observed.

#### COMMENT

Most investigators seem to focus their attention on two types of arteriosclerotic alteration. The first is atherosclerosis or atheroma, which usually affects the larger cerebral arteries and is characterized by patchy degeneration of the intima with local reaction of the media. There occurs a deposition of calcium in the involved portion of the vessel wall. Yellow nodules are found both on the outer and inner surfaces of the arteries in the circle of Willis; these may cause considerable narrowing or complete occlusion of the vessel lumen. Occasionally fatty degeneration of the intima may cause rupture of the inner coat subsequent to which an atheromatous "ulcer" is formed. The damage to the lining endothelium may lead to thrombus formation.

The second type of arteriosclerotic vessel change is less well defined and involves the medium-sized arteries and arterioles and is described as "hyperplastic sclerosis" or "arteriolosclerosis." Histologically, this process is characterized by diffuse thickening of the *entire* vessel wall with a corresponding decrease in the size of the lumen. There is usually considerable hypertrophy of the media and hyperplastic thickening of the intima, and the latter is usually associated with a reduplication of the elastic membrane. In addition there is considerable proliferation of fibrous tissue which gradually replaces the media and adventitia. Degenerative changes of the intima such as hyalinization or fatty degeneration are quite common.

In spite of the vague morphologic resemblance between the so-called "hyperplastic sclerosis" (the second type of arteriosclerosis) and the alterations described in this study, there are significant differences between the two conditions. These might be summarized as follows: (1) The vascular alterations in hyperplastic sclerosis involve the entire vessel wall, including media and adventitia. In the lesions described in the present study the essential changes are confined to the inner layer of the vessel wall. The media and the adventitia did not reveal any marked pathologic alteration. (2) Although intimal pro-

liferation occurs in both conditions, in "hyperplastic sclerosis" this proliferation is usually associated with degenerative changes such as hyalinization or fatty degeneration, changes which were not observed in the cases included in this study. (3) Marked cellular proliferation of the subendothelial connective tissue, a frequent observation in the early stage in my cases, has not been described in hyperplastic sclerosis.

It seems proper to conclude that the vascular lesions described in this study represent a characteristic type of sclerosis of the small cerebral arteries, which can be differentiated without difficulty from hyperplastic sclerosis or from the so-called arteriosclerosis. It is proposed to designate this vascular alteration as "obliterative arteriosclerosis." This term indicates that the main pathologic process consists of a tremendous expansion of the intima, the effect of which is an almost complete obliteration of the vessel lumen.

Confusion with thrombo-angiitis obliterans (Buerger's disease<sup>2</sup>) does not seem likely. In spite of the apparent similarity of the two conditions, the following differential features should be noted: (1) In thrombo-angiitis obliterans the proliferative process of the intima is usually associated with thrombus formation; this is not a common occurrence in "obliterative arteriosclerosis." (2) In thrombo-angiitis obliterans the proliferative changes of the intima are seldom complicated by typical arteriosclerotic changes such as splitting or reduplication of the elastic membrane, findings frequently present in "obliterative arteriosclerosis." (3) The intramural hemorrhages frequently observed in the early stage of thrombo-angiitis obliterans<sup>3</sup> have not been seen in the vascular alterations described in this study.

It is generally known that a certain degree of reactive intimal proliferation may take place in periarteritis nodosa.<sup>4</sup> Confusion with "obliterative arteriosclerosis" does not seem likely; severe inflammatory changes of the entire vascular wall associated with necrosis of the subendothelial connective tissue and adjacent media, characteristic of periarteritis nodosa, were not observed in lesions described in the present study.

Von Glahn and Pappenheimer<sup>5</sup> described specific lesions of peripheral blood vessels in 10 cases of rheumatic cardiac disease. Although in the chronic stage the vascular alterations may be similar to those of obliterating endarteritis, there are significant differences between the two conditions. The vascular lesions described by Von Glahn and Pappenheimer are characterized by exudation of fibrin into and about the blood vessel, by destructive changes in the cellular components of the entire vessel wall, and finally by a distinctive cellular

reaction in the adjacent tissue. None of these alterations were observed in the vascular changes described in the present study.

Lesions similar to those described by Von Glahn and Pappenheimer<sup>5</sup> were reported by Karsner and Bayless<sup>6</sup> in the internal organs, and by Gross, Kugel, and Epstein<sup>7</sup> for the myocardial arteries. Similar vascular alterations of the cerebral blood vessels were described by Bruetsch.<sup>8</sup> The vascular changes were chiefly confined to the meningeal and cortical vessels and were characterized by proliferation of endothelial cells, leading to partial or complete occlusion of the vessel lumen. In addition fibrin plugs were observed into which endothelial cells were growing. In each instance these changes bear very little resemblance to "oblitative arteriosclerosis." The absence of endothelial proliferation and fibrin plugs in the cases described in this study is sufficient to exclude vascular lesions of this category.

Obliterative arteritis is frequently seen with syphilis of the brain. It is well known that in cases of syphilis the intima may have proliferated and become thickened, giving the appearance of endarteritis. However, the differentiation from "oblitative arteriosclerosis" is not difficult. In syphilitic arteritis all three layers of the blood vessel are affected; the adventitial tunic and its spaces are infiltrated with lymphocytes and plasma cells, the adventitial cells themselves having proliferated. None of these changes are present in "oblitative arteriosclerosis."

#### SUMMARY

Distinctive alterations of the smaller blood vessels as found in the brains of 10 cases with cerebral arteriosclerosis are described. This lesion is characterized by tremendous expansion of the intima, resulting in narrowing or complete obliteration of the vessel lumen. It is proposed that this process be designated as "oblitative arteriosclerosis," and considered as a special type of arteriosclerosis of small cerebral blood vessels. Emphasis is placed on differentiation from "hyperplastic sclerosis."

Histologic changes in the parenchyma of the brain, particularly the cortical gray matter, consisted of diffusely scattered, stripe-like, small, old and recent softenings secondary to the oblitative vascular lesions.

A gross finding in the brain which was regarded as characteristic of "oblitative arteriosclerosis" was a granulated appearance of the cortical surface, due to numerous focal areas of glial scarring, often associated with stripe-like areas of softening involving the upper layers of the cortical ribbon.

"Oblitative arteriosclerosis" may occur independently of arteriosclerotic changes of the major cerebral arteries.

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[ Illustrations follow ]



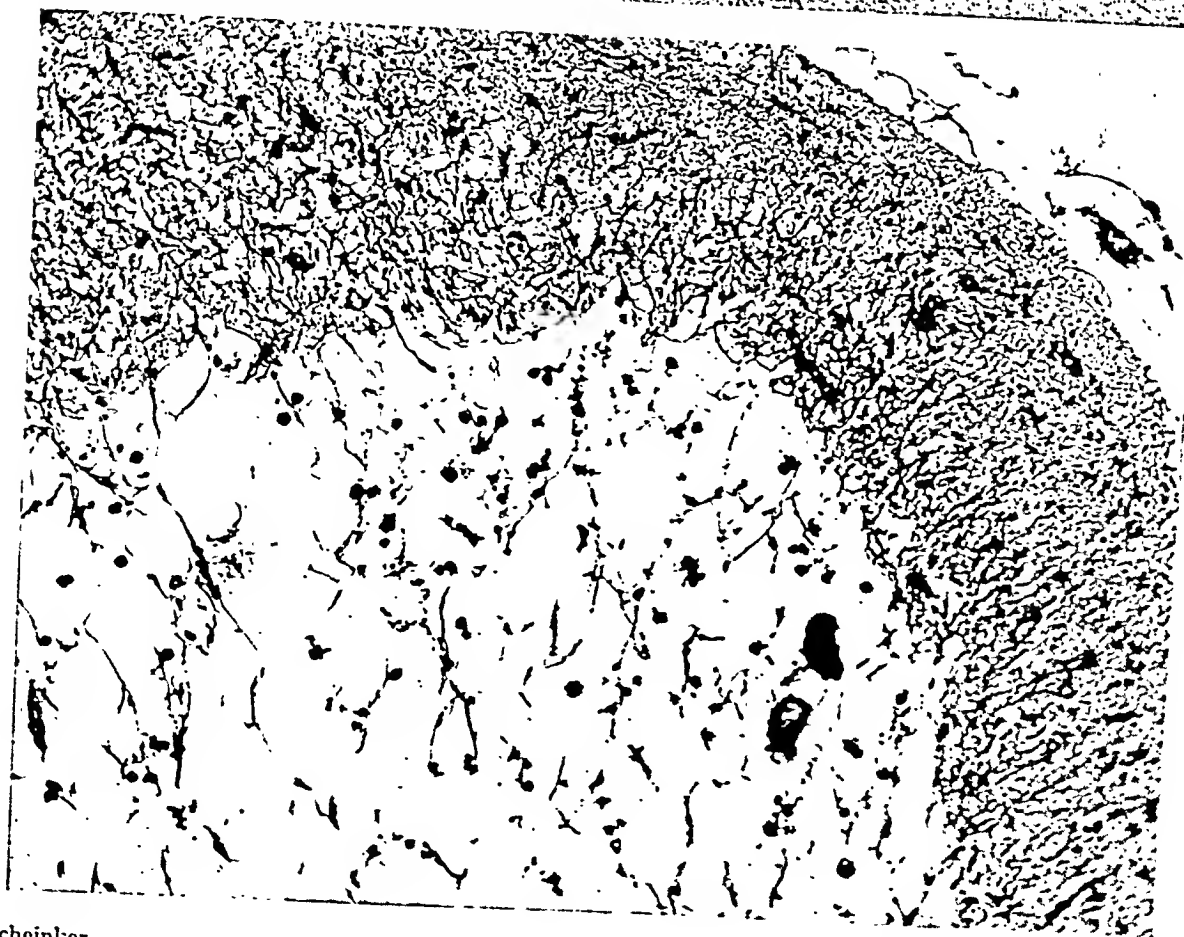
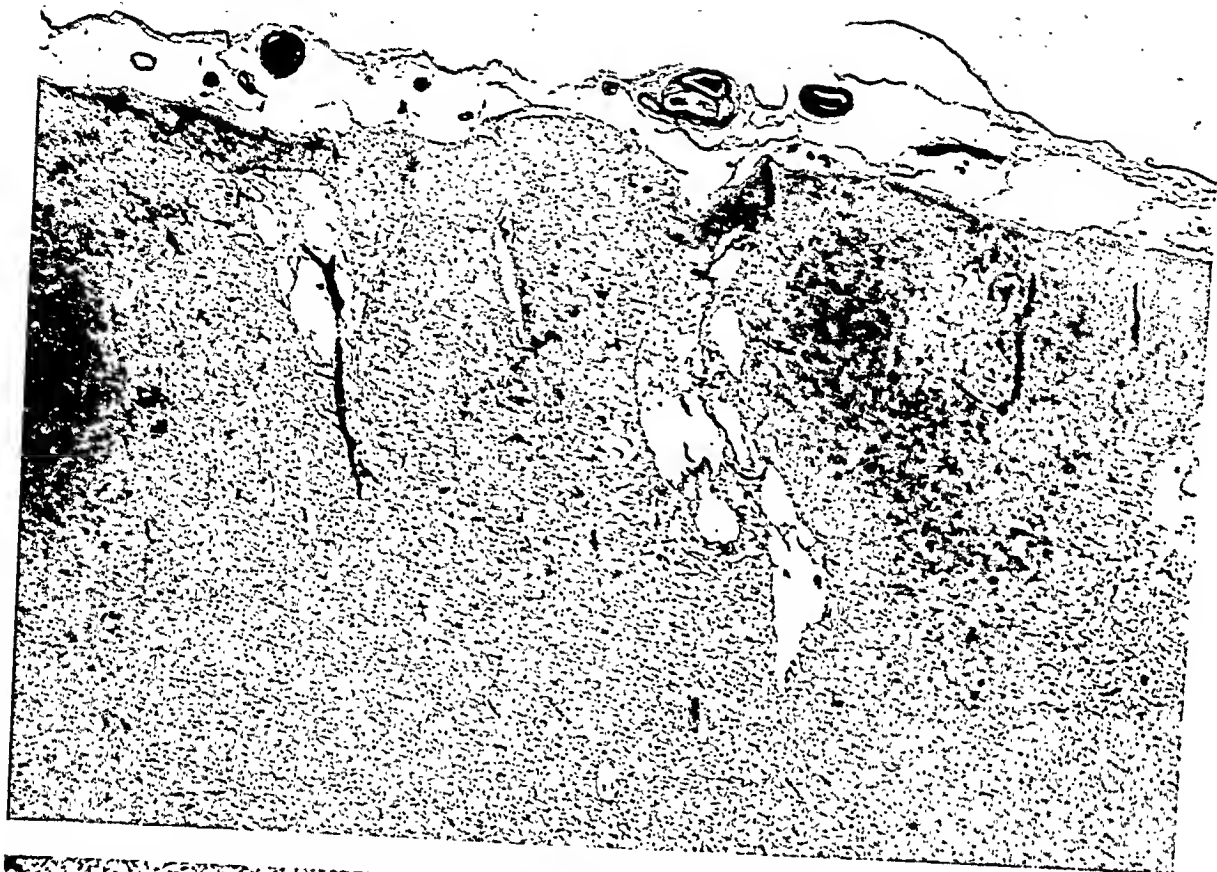
## DESCRIPTION OF PLATES

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### PLATE 118

FIG. 1. Numerous small foci of cystic softening and glial scar formation diffusely scattered throughout the upper layers of the cortical ribbon, producing a granulated appearance of the cortical surface. Hematoxylin and eosin stain.  $\times 60$ .

FIG. 2. Small area of cystic softening of the cortical gray matter, containing numerous gitter cells. Cresyl violet stain.  $\times 115$ .



Scheinker

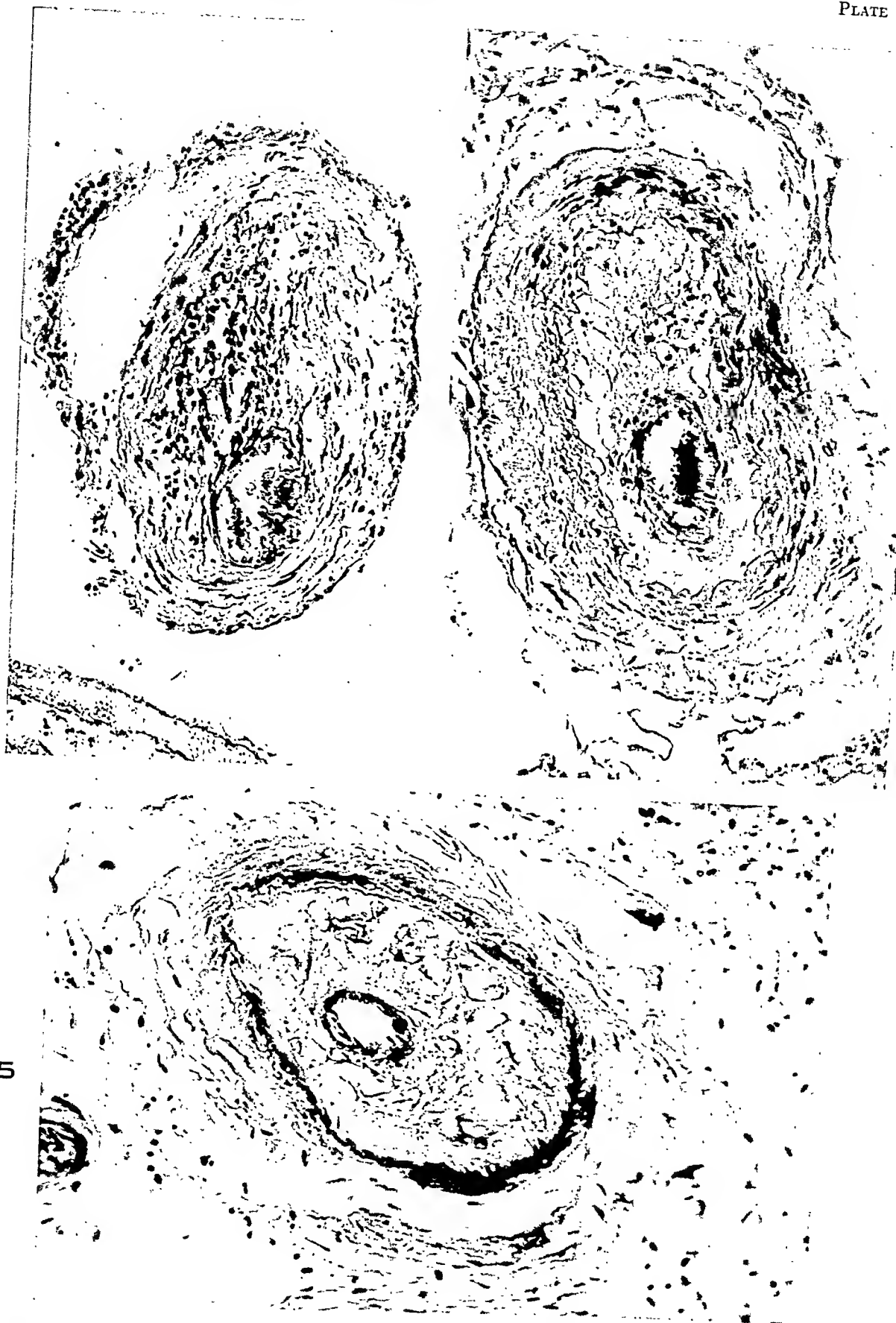
Obliterative Cerebral Arteriosclerosis

PLATE 119

FIG. 3. Early proliferative stage of the characteristic lesion, confined chiefly to the inner layer of a small artery. Hematoxylin and eosin stain.  $\times 115$ .

FIG. 4. Small meningeal artery with a considerably reduced lumen resulting from an expansion of the ground substance of the subendothelial connective tissue. The media and adventitia are relatively well preserved. Hematoxylin and eosin stain.  $\times 130$ .

FIG. 5. Advanced necrobiotic stage of the lesion with tremendous expansion of the intima, and consecutive, almost complete, obliteration of the vessel lumen. Hematoxylin and eosin stain.  $\times 130$ .



Scheinker

Obliterative Cerebral Arteriosclerosis.

PLATE 120

FIG. 6. Two small arteries with tremendous thickening of the intima. The lumina are reduced to mere slits. Hematoxylin and eosin stain.  $\times 165$ .

FIG. 7. A small meningeal artery with a tremendous expansion of the subendothelial connective tissue and almost complete obliteration of the lumen. Hematoxylin and eosin stain.  $\times 165$ .



Scheinker

Obliterative Cerebral Arteriosclerosis



# THE CENTRAL NERVOUS SYSTEM IN PNEUMONIA (NONSUPPURATIVE PNEUMONIC ENCEPHALITIS)

## II. A PATHOLOGIC STUDY \*

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Although an abundant literature has accumulated on the subject of pneumonia, almost none of the published articles have considered the possible complications of the nervous system in this disease. This omission suggests the indifference with which this extremely important neurologic condition has been handled and indicates that perhaps many cerebral manifestations in pneumonia are being overlooked. In spite of the numerous organisms that are capable of producing a pneumonia, the clinical picture of the cerebral complications appears to be fairly constant (Baker and Noran<sup>1</sup>). During the acute stage of the pneumonitis there may occur generalized complaints such as headache, vomiting, diplopia, and lethargy (Eschbach,<sup>2</sup> Reimann,<sup>3</sup> Bonaba, Marcos, and Mendivil de Agorio,<sup>4</sup> Gareiso and Sagreras<sup>5</sup>). Occasionally, the involvement of the nervous system is much more dramatic and is manifested by coma, convulsions, or severe delirium often associated with visual hallucinations (Eschbach,<sup>2</sup> Comby,<sup>6</sup> Stephan,<sup>7</sup> Navarro,<sup>8</sup> Piaggio Garzón,<sup>9</sup> Bernheim and Bonnefoy,<sup>10</sup> de Filippi and Fernández,<sup>11</sup> Nové-Josserand, Rougier, and Feuillade,<sup>12</sup> Prandi<sup>13</sup>). Most of these symptoms have been assumed to be secondary to pyrexia even though they may occur in mild cases in which the temperature has not been significantly high. Sporadic cases have been reported in the foreign literature in which varying neurologic complications have appeared during convalescence while the temperature was normal and the patient was assumed to be well on the road to recovery (Gareiso and Sagreras,<sup>5</sup> Comby,<sup>6</sup> Piaggio Garzón,<sup>9</sup> Nové-Josserand *et al.*,<sup>12</sup> Lesieur, Froment, and Garin,<sup>14</sup> Regine,<sup>15</sup> Barni,<sup>16</sup> Grenet, Isaac-Georges, and Desmarquest,<sup>17</sup> Mouriquand, Bernheim, and Boucomont<sup>18</sup>). In many of these patients the cerebral involvement left permanent residua in the form of motor weakness, ataxia, aphasia, and athetosis, or were severe enough to result in cerebral death (Reimann,<sup>3</sup> Stephan,<sup>7</sup> Piaggio Garzón,<sup>9</sup> Mouriquand *et al.*<sup>18</sup>).

While clinical reports are relatively few, detailed pathologic investigations of this condition are even more conspicuous by their absence. A few scattered case reports are available and in these the

\* Aided by a grant from the University of Minnesota Graduate School.

Received for publication, June 13, 1945.



cerebral changes are mentioned only briefly. Guillain and Vincent,<sup>19</sup> and Fraenkel<sup>20</sup> emphasized the presence of pneumococci around scattered vessels. Fraenkel noted that tiny cerebral vessels had undergone endothelial proliferation resulting in vascular occlusion. Perivascular erythrocytes and leukocytes were also observed. Mollard and Dufourt<sup>21</sup> mentioned petechiae in one of the their fatal cases. Adler,<sup>22</sup> in a study of 5 fatal cases of pneumonic encephalitis, reported changes varying from mild congestion to extensive perivascular infiltrations of leukocytes, hemorrhages, and even demyelination. The cases with the more severe tissue changes were called "proliferative pneumonia encephalitis" while those with mere congestion or hemorrhage were designated as "toxic-pneumonia encephalitis." Perrone and Wright<sup>23</sup> observed small hemorrhages, perivascular cuffs of mononuclear cells, and an astrocytic proliferation in the brain of a patient who had virus pneumonia. Golden<sup>24</sup> and Ingleby<sup>25</sup> described similar lesions in cases of encephalitis secondary to atypical pneumonia, presumably due to a virus. Golden favored the term, hemorrhagic encephalopathy. Ingleby appears to have been the only investigator to report inclusion bodies in the brain.

The more complete pathologic studies have been those by Lépine,<sup>26</sup> Bonaba and Barberousse,<sup>27</sup> and Marinesco, Jonesco-Sisesti, and Stroesco.<sup>28</sup> In a 53-year-old male who developed hemiplegia following pneumonia, Lépine observed a single area of softening involving the left middle frontal gyrus. No microscopic studies were performed. Bonaba and Barberousse described in detail the cerebral alterations in a 12-year-old child who manifested convulsions during the acute phase of a pneumonia. At autopsy the most prominent changes were found within the basal nuclei, where there occurred marked ependymal proliferation and diffuse glial increase. There were some perivascular infiltrates forming incomplete collars around the vessels. Vascular congestion and hemorrhage were most prominent within the putamen. Scattered nerve cells within the basal nuclei showed acute changes consisting of chromatolysis and swelling. The cerebral cortex and white matter were surprisingly free of lesions. Marinesco and his co-workers reported very complete histologic studies in 3 cases of pneumococcus encephalitis. The lesions consisted of scattered plaques of demyelination which predominated in the subcortical and periventricular white matter. These plaques were often perivascular with the zone of tissue directly adjacent to the vessel being best preserved, giving the area of demyelination a ring-like arrangement. The neuroglia within these demyelinated areas was destroyed while

at the periphery a mild gliosis had occurred. There was a mild diffuse astrocytic proliferation throughout the white matter. Vascular changes were also very prominent, particularly in the involved areas. Many of the smaller vessels contained fibrin thrombi with intermixed red and white cells. Numerous vessels were surrounded by accumulations of red or white cells. The spinal cord showed a marginal demyelination but its nerve cells appeared intact.

Even though the sporadic reports recorded in the literature leave little doubt concerning the existence of a pneumonic encephalitis, the paucity of complete pathologic studies has left numerous gaps in our knowledge of the morphologic nature of the lesions. It was felt, therefore, that a careful study of the histopathologic findings in this condition was definitely indicated.

We have been able to collect the brains from 10 cases of pneumonic encephalitis in which complete autopsies were performed. In all instances there was unquestionable evidence of encephalitis established by histologic study. Since we are primarily interested in the pathologic alterations within the nervous system, an extensive consideration of the clinical manifestations does not seem indicated. However, the salient clinical features, the anatomic variety of pneumonia, and the nature of the etiologic agent are presented in table form (Table I).

Most of our cases occurred in infants, but no age group was excluded. Three patients were past middle age, being 45, 50, and 50 years of age. There was no preference for either sex. In infancy the symptoms resulting from the pneumonia were often mild and the cerebral manifestations tended to be rather late in developing, at times appearing after recovery from pneumonitis had begun. Such a sequence was well demonstrated in case 3. In half of the cases the clinical evidence of involvement of the nervous system seemed mild. In one case (case 2) encephalitis was completely overlooked clinically even though the microscopic examination of the brain left no doubt about the existence of definite cerebral involvement.

The lungs were examined carefully in all cases, but a detailed account of the microscopic findings is not pertinent to this study. From the anatomic standpoint there were 5 cases of broncho-, lobular, or interstitial pneumonia, 3 cases of a typical lobar involvement, and 2 examples of diffuse hemorrhagic pulmonary lesions showing associated edema and consolidation.

The etiologic agent varied. Four cases were characteristic of the recently popularized, atypical pneumonia, which is presumably of viral origin. In 4 others definite bacterial agents were found: nonhemolytic

TABLE I  
Summary of 10 Cases with Encephalitis Associated with Pneumonia

Case	Age and sex	Clinical features	Encephalitic manifestations	Anatomic type of pneumonia	Etiologic agent of pneumonitis
1. H.F.	Male 6 months	Cough, low-grade fever, transitory mild empyema and otitis; sulfathiazole without response for a brief period only	Irritability and restlessness	Bronchopneumonia (largely resolved); healed pleurisy; thrombosis of the small pulmonary arteries	Staphylococcus, coagulase positive
2. Baby L	Female Newborn	Irregular respiratory distress and fever; gradual failure and death after 1 month	None noted on chart	Hemorrhagic consolidation of both lungs	Lipoid pneumonia (established by sudan III staining)
3. Baby H.	Male 26 days	Cough, fever, pneumonia, respiratory difficulty	Convulsive phenomena, stupor, and death	Bronchopneumonia (largely resolved)	Virus(?)
4. E.C.	Male 50 years	Acute febrile state and pneumonia	Choked disks	Extensive lobar pneumonia	Streptococcus, nonhemolytic
5. F.P.	Male 50 years	Clinical lobar pneumonia; fulminating course	Delirium; nuchal rigidity	Bilateral hemorrhagic pneumonia	Streptococcus, nonhemolytic
6. A.F.	Female 1½ mo.	Cough, slight intermittent fever, thrush of nose and throat; death in respiratory failure	Moderate lethargy	Bilateral interstitial pneumonia; no thrush involving bronchial tree	Virus(?) (thrush of nose and throat)
7. A.F.	Male 4½ mo.	Mild pharyngitis, cough	Spinal fluid: pleocytosis of 50 cells	Pneumonia, lobular	Not determined
8. Q.	Male 2 months	Cough, cyanosis, and fever	Apathy, listlessness, and general flaccidity	Pneumonia, lobular	Virus(?)
9. P.	Female 10 days	Rhinitis, mild irregular fever, cough, dysphagia and cyanosis	Lethargy	Bronchopneumonia	Virus(?)
10. E.A.	Female 45 years	Fever, cough, and pulmonary findings	Stupor, coma, muscular twitching, positive Babinski signs, and increased spinal pressure	Lobar pneumonia	Pneumococcus (type VIII)

streptococcus (2), staphylococcus, and type VIII pneumococcus. Probably the most remarkable case of encephalitis was that following a typical lipoid pneumonia confirmed by sudan III preparations.

In spite of the great variation in the severity and in the cause of the pneumonia, we found that the pathologic picture of the encephalitis was similar in all. The histologic findings in the various cases differed only in degree. For this reason it is possible to combine the observations from the entire group into one composite pathologic description. In all cases samples of tissue were taken from a number of scattered areas of the brain for microscopic examination. Each block was prepared by means of the following technics: hematoxylin and phloxine stain, Nissl (cresyl violet) stain, Weil stain for myelin, phosphotungstic acid hematoxylin stain for glial fibers and fibrin, Bodian stain for neurofibrils and axis cylinders, and Weigert-van Gieson method for elastic fibers, connective tissue, and smooth muscle.

### PATHOLOGIC ALTERATIONS

On gross examination the external surface of the brain invariably showed a marked vascular congestion and usually an associated, patchy, brownish discoloration resulting from irregular, small, subarachnoid hemorrhages. In certain instances rather large areas of the cerebral hemispheres were obscured by subarachnoid hemorrhage. Coronal sections of the brain consistently demonstrated prominent congestion. In the more severe encephalitic involvement, large numbers of petechiae were disseminated throughout the entire cerebrum with a predilection for the subcortical white substance. In an occasional case, these petechiae occurred only in more or less localized portions of the brain and were most prominent in the cortical gray matter.

### MICROSCOPIC OBSERVATIONS

Microscopic study of the nervous system in pneumonic encephalitis revealed a uniform pathologic picture except for differences in severity. Such variations were readily correlated with the clinical severity and duration of the encephalitic complication. In order to arrive at a better understanding of the morphologic features, the anatomic changes observed in the various stages of this illness will be described under the following headings: those of mild, moderately severe, and most severe pneumonic encephalitis.

#### *Mild Encephalitis*

Even the early and milder cases revealed very extensive thrombosis of the cerebral vessels. This finding constituted the most significant

pathologic feature, and often was the only microscopic manifestation. Vessels without thrombi were distended with blood. (Because perivascular hemorrhages and secondary parenchymal changes are generally absent at this stage, this thrombotic process frequently is overlooked and is regarded merely as post-mortem clotting.)

The small vessels, arterioles, venules, and capillaries, showed by far the most frequent thrombotic involvement. The process of vascular thrombosis could be traced readily through the various stages of its development. The earliest evidence of thrombus formation was the observation of platelets, fibrin, or erythrocytes adhering to the intimal lining of a cerebral vessel. Later, the marginal zone of the vessel was obliterated, leaving only a small central opening through which red blood cells continued to circulate (Fig. 1). This remaining central channel of the blood stream was steadily narrowed by the peripheral clotting until the vessel was completely obstructed.

Either before or shortly after the clotting completely obstructed the lumen, the erythrocytes within the thrombus quickly lost their individual contours, producing a homogeneous mass. In many sections the thrombi were all of a uniform homogeneous appearance with none of the structural components visible (Fig. 1).

As a rule, in either the partial or the complete vascular occlusions, only a few fine fibrils of fibrin were visible within the thrombus when only the routine hematoxylin-phloxine stain was used. However, with special staining a rather rich network of fibrin was usually demonstrated throughout the thrombus (Fig. 2).

Hemorrhagic lesions were very mild and infrequent. Sparse erythrocytes were seen in the perivascular spaces of only a small number of scattered vessels. An extravasation of blood sufficient to produce a notable filling of the Virchow-Robin space was very unusual. Although a mild, patchy, subarachnoid hemorrhage was common, no more than a few erythrocytes were observed within the leptomeninges. The walls of the cerebral vessel showed no degenerative or proliferative changes and displayed no involvement by inflammatory cells. A microglial reaction did not occur.

In general, the ganglion cells appeared normal but occasionally a few neurons revealed a mild swelling and chromatolysis. The myelin was essentially normal, showing only a very minimal perivascular degeneration around a few of the thrombosed vessels. There was no astrocytic proliferation.

#### *Moderately Severe Encephalitis*

In the more involved cases, thrombosis became even more extensive, implicating a relatively large percentage of the cerebral vessels of all

sizes, with either partial or complete occlusion of their lumina. The structure of most thrombi was essentially the same as that described in the milder cases except that more fibrin was usually present. Very few leukocytes were found in the thrombi. In the occlusions of the larger vessels, prominent collections of platelets were often detected within the thrombi, producing a somewhat lamellated appearance. In these larger vessels a multiplicity of branching threads of fibrin were frequently visible without the use of special stains. There was no organization of thrombotic material.

Perivascular hemorrhages became numerous. Many of these extended into the neural tissue in the vicinity of the perivascular spaces, producing ball petechiae. A small number of ring hemorrhages also developed. Diffuse intracerebral bleeding did not occur. A thick layer of blood might be found distending the subarachnoid space over large regions of the brain surface. A sparse, secondary, leukocytic exudate might occasionally be associated with the subarachnoid bleeding.

There were no changes in the blood vessel walls to account for the thrombosis and hemorrhage. In one of our cases proliferative changes occurred in the walls of several vessels in the region of the basal nuclei. Generally a few leukocytes, consisting of a mixture of polymorphonuclear neutrophils and mononuclear cells, were scattered irregularly around the adventitia of a small number of vessels (Fig. 3).

Many nerve cells throughout the gray matter of the brain revealed a marked tigrolysis and often a concomitant alteration of the cell nucleus. Only a few ghost cells resulted.

Mild alterations of the myelin sheaths were observed. The most characteristic involvement consisted of a narrow perivascular zone of swelling and a mild rarefaction of the myelin sheaths. Discrete perivascular foci of advanced demyelination were rare (Fig. 4). A few of these lesions occurred in only one case in this series. Usually there were a few areas of diffuse demyelination within the cerebral white substance. In some of the regions of myelin degeneration, a slight proliferation of the microglia occurred. This microglial reaction consisted principally of small microgliocytes intermixed with a small number of red cells and fat-granule elements. A few scavenger cells were occasionally found in the adventitia of certain vessels infiltrated by leukocytes. The macroglia showed no evidence of activity.

#### *Very Severe Encephalitis*

The advanced degree of cerebral alteration occurred in only rare instances of pneumonic encephalitis. In our material only one case revealed this severe form. Thrombus formation was still the most striking pathologic finding. An even greater number of vessels of all

sizes were thrombosed. Many of the thrombi were similar to those previously described. However, a multiplicity of small vessels were filled with eosinophilic staining homogeneous material which resembled fresh platelet thrombi. Special stains, however, demonstrated that the thrombotic substance was comprised largely of coils of very coarse fibrin which tended to fuse into an almost solid mass (Fig. 5). In an occasional instance, fine fibers of fibrin extended outside the vessel to invade the perivascular parenchyma (Fig. 5).

Perivascular hemorrhages became more numerous and were of both the ball and ring types (Fig. 6). These perivascular extravasates frequently encircled small thrombosed vessels but the central thrombus was usually visible in only the ring petechiae, since the extravasated blood itself obscured the central vessel of the ball hemorrhage (Fig. 6). Prominent focal collections of mixtures of polymorphonuclear and mononuclear leukocytes were found in the centers of a small number of petechiae of both the ball and ring types. A few scavenger cells might occasionally be intermixed with the leukocytes.

Neurocellular alterations were very frequent and quite severe throughout the brain. The most striking finding consisted of marked shrinkage, pyknosis, and irregularity of the cell bodies of many of the neurons. Some cells were slightly swollen and stained very lightly to appear as ghost cells. A few were completely destroyed. In the deeper layers of the cortex there frequently occurred a disturbance of the polarity and lamination of the neurons, resulting in a wind-blown appearance of the involved areas (Fig. 7).

The myelin showed a more severe degenerative change. Small patchy areas of swollen and rarefied myelin sheaths were numerous, especially in the subcortical white matter and the centrum ovale (Fig. 8). A more marked and diffuse demyelination sometimes occurred in the vicinity of a large collection of petechiae. In these situations vacuolization and partial fragmentation of the neural tissue occasionally took place. Even in the areas of more severe myelin degeneration, only mild microglial proliferation occurred, associated with very few gitter or scavenger cells. The axis cylinders showed very little alteration even in these cases with severe involvement, the only exception being the destruction of axons in the areas directly involved by focal hemorrhage. A notable degree of astrocytic proliferation did not occur.

#### DISCUSSION

A very important result of this investigation was the observation that essentially identical pathologic lesions of the nervous system occur in all cases of pneumonic encephalitis, even though the etiologic

agent of the pneumonia varies greatly from case to case. The earliest encephalitic alterations consisted of diffuse congestion and thrombosis of a large number of the smaller cerebral vessels. Mild perivascular and subarachnoid bleeding soon developed. There was very little cellular inflammation, myelin degeneration, or nerve cell change.

In the more severe encephalitic complications many thrombosed vessels and hemorrhagic lesions were observed. The latter consisted of ring and ball petechiae as well as abundant subarachnoid bleeding. The thrombi involved many of the larger vessels as well as a majority of the smaller ones, and most of them contained abundant fibrin. Perivascular inflammatory elements were also somewhat more frequent. Nerve cell disease was moderately severe throughout the brain, resulting in occasional areas of neuronal devastation. Myelin sheath changes were more prominent but tended to remain quite mild. Although we have not had the opportunity to study the pathologic changes within the nervous system in the more chronic cases, we have been able to follow several patients clinically for more than a year. The fact that the symptomatology seemed progressive for many months suggested the presence of a very extensive involvement of the brain. Such a deduction, however, must be proved by future investigations.

The actual causative agent of the encephalitis has not been identified. Many theories are available which might be used in explaining this form of cerebral complication. These include: (1) pyrexia, (2) toxemia secondary to the pneumonia, (3) direct invasion of the brain by the bacterial agent causing the pneumonitis, (4) generalized virus infection involving the brain as well as the lungs, (5) activation of a latent filterable virus previously lying dormant within the nervous system, (6) drug hypersensitivity in cases treated with serum or sulfonamides, (7) anoxemia, and (8) allergic reaction.

The observation that encephalitis may develop in mild, afebrile cases, showing no signs of any notable degree of toxemia, and in patients already convalescing from their pneumonia seems sufficient to exclude pyrexia and toxemia as the causative factors. The paucity of inflammatory elements speaks against a direct bacterial invasion of the nervous system. Ingleby<sup>25</sup> found numerous inclusion bodies within the brain, lungs, and other viscera in cases of pneumonic encephalitis. She, therefore, postulated a virus as the causal factor for her cases. However, in other cases of encephalitis complicating the typical virus pneumonia, inclusion bodies have not been found (Perrone and Wright<sup>23</sup> and Golden<sup>24</sup>). Moreover, the pathologic changes within the central nervous system show no resemblance to those lesions described in the usual virus form of encephalitis.



Many of the so-called postinfectious encephalitides have been attributed to the activation of a latent neurotropic virus. The constantly uniform pathologic manifestations of pneumonic encephalitis make it necessary for one to consider this theory carefully. However, instead of the usual foci of perivascular demyelination observed in the postinfectious encephalitides, the pneumonic complication is characterized by thrombosis of many vessels and scattered hemorrhagic lesions, thereby disproving the possibility of any etiologic similarity between these two conditions.

A drug sensitivity has been implicated as the cause of certain cases of periarteritis nodosa and has been verified experimentally for the sulfonamide drugs by Rich and Rich and Gregory.<sup>29</sup> None of our patients were given serum and in the few who received sulfonamide drugs there was no apparent relation between the administration of the drug and the onset of cerebral-symptoms.

An allergic reaction to the primary infectious condition frequently has been suggested as the cause of different types of postinfectious encephalitis. Ferarro<sup>30</sup> produced cerebral lesions experimentally that were very similar to those of scarlatinal encephalitis. The fact that either foreign proteins or brain extracts of a heterologous animal were used as the antigens in his experiments casts doubt upon such a hypothesis as the explanation for the lesions observed in the present study. However, this allergic theory cannot be discarded without further investigation.

Golden<sup>24</sup> believed that the encephalitic involvement in cases of pneumonia might be the result of anoxemia. This concept seems very unlikely since encephalitis often occurs in cases without any clinical evidence of oxygen want and also after the pneumonitis has largely resolved. Since cyanosis and respiratory distress occur with only minimal lung involvement, it is possible that the symptoms suggesting anoxemia may actually be central rather than pulmonary in origin. The multiplicity of thrombosed cerebral vessels in pneumonic encephalitis should be adequate to differentiate this condition from anoxia.

The occurrence of uniformly identical cerebral alterations in all of our cases indicates that pneumonic encephalitis most likely has a constant pathogenesis and is not directly related to the etiologic agent of the pneumonia, which may be one of various bacteria, viruses, or even lipid. The only constant factor in these cerebral complications is the nonspecific inflammatory process within the lungs. Therefore, it seems logical to infer that in the pulmonary tissue itself may be found the primary cause of the encephalitis.

Since the most characteristic pathologic manifestation of this variety

of encephalitis is a very extensive thrombosis, we have felt that perhaps the basis of this illness may be related in some way to disturbance in the clotting mechanism. We would like to suggest further that some factor from the lung tissue itself may gain access to the circulation under pathologic conditions and thus initiate the abnormal intravascular clotting observed within the nervous system. Experiments now under way seem to substantiate this concept.

### SUMMARY

1. A survey of the literature regarding pneumonic encephalitis reveals only a small number of sporadic clinical and pathologic case reports.

2. A careful study of the pathologic lesions of the brain in 10 cases of pneumonic encephalitis revealed that the cerebral alterations are uniform throughout the entire series, even though the cause of the pneumonitis is highly variable.

3. Extensive thrombosis and prominent perivascular hemorrhages are the outstanding microscopic findings observed in the nervous system.

4. Various theories regarding the pathogenesis of this type of encephalitis have been presented. The prodigious number of thrombosed cerebral vessels observed in this study suggests the possibility that some alteration in the clotting mechanism of the blood may cause these cerebral lesions.

5. The constancy of the cerebral lesions, regardless of the type of pneumonia, indicates that the real cause of the encephalitis may be the pulmonary tissue itself. Some factor from the lung parenchyma may possibly accelerate intravascular clotting.

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[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

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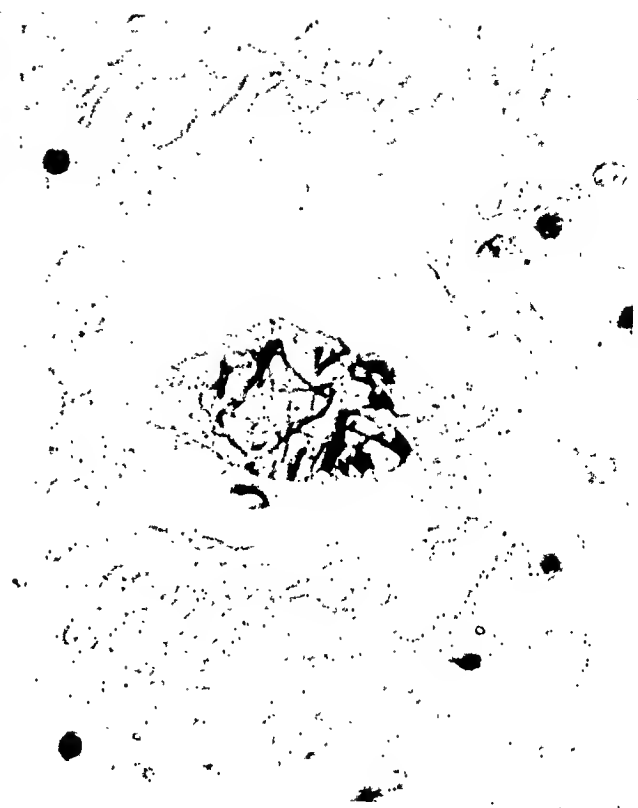
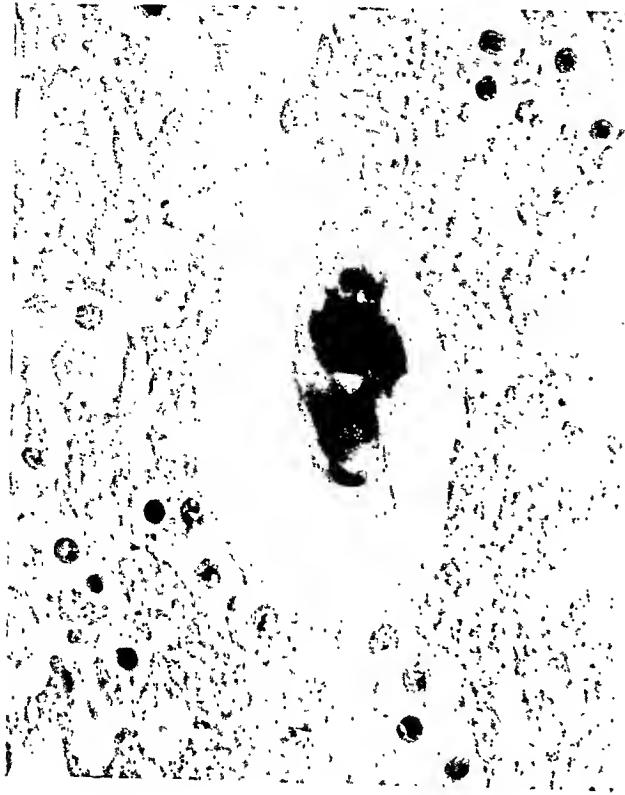
### PLATE 121

FIG. 1. There is a partial obliteration of the vessel lumen by pale homogeneous thrombotic material. Red blood cells continue to circulate through a narrowed channel. Hematoxylin-phloxine stain.  $\times 460$ .

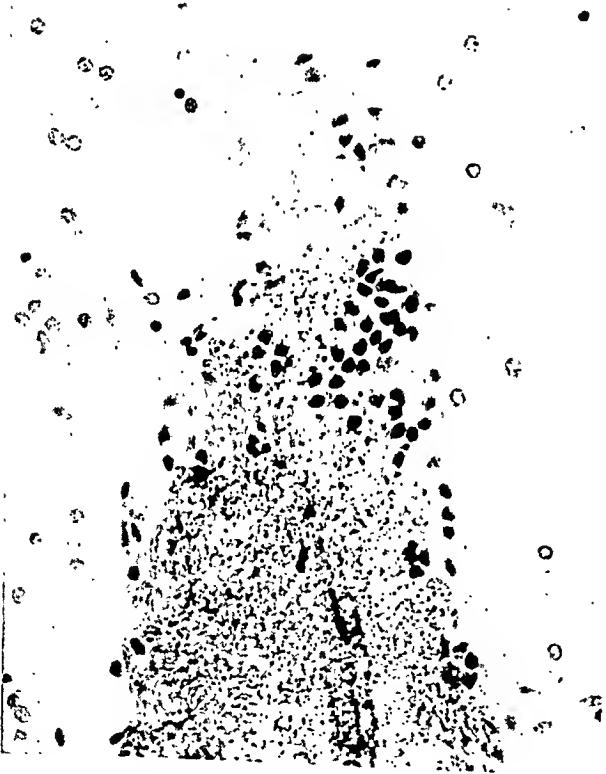
FIG. 2. The vascular lumen is filled with abundant fibrillar fibrin. Mild rarefaction and swelling of the myelin around the vessel is evident. Phosphotungstic acid hematoxylin stain.  $\times 460$ .

FIG. 3. Sparse collections of neutrophils and mononuclear leukocytes are scattered irregularly along the adventitia of the vessel. There are only very few microglial elements. Nissl stain.  $\times 460$ .

FIG. 4. This section reveals a prominent perivascular focus of demyelination. Hematoxylin-phloxine stain.  $\times 140$ .



2



3



4

Noran and Baker

Encephalitis Associated with Pneumonia

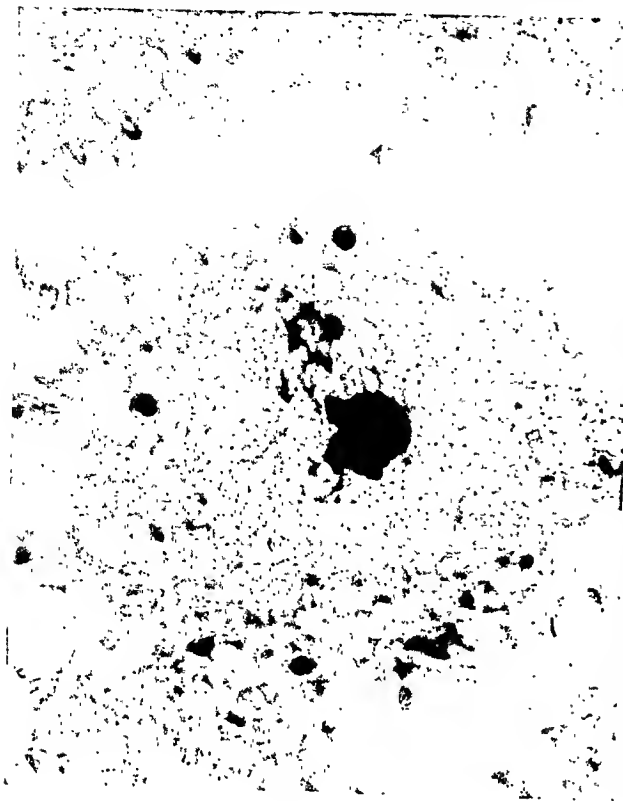
PLATE 122

FIG. 5. A ring petechial hemorrhage is illustrated. Coarse coils of fibrin occur within the central vessel with fine threads of fibrin extending into the necrotic perivascular parenchyma. Phosphotungstic acid hematoxylin stain.  $\times 460$ .

FIG. 6. This section reveals three ring and one ball type of hemorrhage. There is a thrombus in the center of each ring hemorrhage. Weil stain.  $\times 45$ .

FIG. 7. Marked changes in the nerve cells are found in this area. Most of the neurons are shrunken and pyknotic. There are a few ghost cells. The polarity of most of the neurons is disturbed. Nissl stain.  $\times 230$ .

FIG. 8. Most of the tissue has undergone myelin degeneration. Many of the focal lesions are perivascular. Weil stain.  $\times 140$ .



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# ADENOMATOID TRANSFORMATION OF THE GLOMERULAR CAPSULAR EPITHELIUM\*

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The following case is of interest because of a unique renal lesion which seems not to have been previously described.

The patient was an Italian-American housewife, 54 years old, admitted to the Presbyterian Hospital because of jaundice of 1 month's duration. In the past her health had been good, despite the fact that her diet had been low in meat and rich in its carbohydrate content. Her family history was irrelevant. Five months before admission she began to have eight to nine watery bowel movements daily. The stools were never bloody, tarry, or foul. One month later anorexia, swelling of the abdomen and ankles, and vague pain in the epigastrium and lower extremities appeared. For 1 month prior to entry the patient had had jaundice, with dark urine, light stools, nausea and occasional vomiting. A pronounced weight loss also had occurred.

On physical examination the patient appeared obese, jaundiced, and chronically ill. Examination of the heart and lungs was negative save for an apical systolic murmur. The liver edge extended to the pelvic brim and was hard, nodular, and not tender. No ascites was evident, but pitting edema of the lower extremities was pronounced.

The laboratory data of interest follow. Urine: specific gravity, 1.005 to 1.018; albumin, 2 plus to 4 plus; glucose on five occasions, 0, once was 1 plus; bile, 3 plus; 5 to 10 white blood cells; rare red blood cells, occasional hyaline and granular casts. Blood: red blood cells, 4,020,000 per cmm.; hemoglobin, 11.6 gm. per cent; white blood cells, 28,000 per cmm. (polymorphonuclear cells, 84 per cent, slight shift to left); platelets, "slightly reduced"; erythrocyte sedimentation rate, 77 mm. per 1 hour; Kline test, negative. Blood serum: alkaline phosphatase, 26.0 Bodansky units per cent; urea nitrogen, 13 mg. per cent; bilirubin, 6.3 mg. per cent; cholesterol, 625 mg. per cent; albumin, 2.8 gm. per cent; globulin, 3.6 gm. per cent; euglobulin, 0.6 gm. per cent; cephalin flocculation, negative; fasting sugar, 89 mg. per cent. Prothrombin time, 26.4 seconds. Stool: guaiac test, 4 plus. Urine: *Streptococcus viridans* and *Staphylococcus albus* obtained by culture. Electrocardiogram, sinus tachycardia. Roentgenogram, suggestion of esophageal varices.

The course was afebrile but her condition grew worse steadily. Obstructive jaundice persisted and there was leukocytosis ranging as high as 42,800 white blood cells per cmm. Finally the patient became stuporous and died on the 39th day following her admission to the hospital.

At necropsy the body was that of a short, obese, deeply jaundiced, middle-aged white female with a moderate amount of pitting edema over the sacrum, abdominal wall, left breast, and both lower extremities. About 250 cc. of thin, faintly turbid, pale, yellow fluid was present in the peritoneal cavity and smaller amounts of similar fluid were found in the pleural cavities. Firm fibrous adhesions bound part of the omentum to the abdominal wall but otherwise the serous surfaces were smooth and glistening. Briefly, the abnormal anatomical find-

\* Received for publication, June 25, 1945.

ings were as follows: chronic cholecystitis; calculi in gallbladder; carcinoma of gallbladder with extension to common bile duct and to liver; secondary carcinoma in lymph node (hepatic), liver, and lung; jaundice; ascites; varices of esophageal veins due to portal obstruction (by neoplastic tissue); splenomegaly; varices of hemorrhoidal veins; extramedullary hematopoiesis in spleen, liver, periadrenal adipose tissue, kidneys, and stomach; ulcer of stomach (acute); cardiac hypertrophy; chronic cervicitis; polyps of cervix and of uterus; fibrous peritoneal adhesions; fibroma of left kidney; hypertrophy of kidneys and proliferation of capsular and tubular epithelium (described below).

The kidneys were alike. Each was considerably enlarged, the right weighing 320 gm. and the left, 300 gm. The capsule stripped readily, revealing a surface which was normal except for the presence of a few shallow scars. On section, the renal tissue was moderately jaundiced. Corticomedullary differentiation was distinct and all markings appeared normal. In a centrally placed pyramid on the left there was a small fibroma. The pelves, calyces, and ureters were normal.

Histological examination revealed that in both kidneys nearly all glomeruli had undergone a striking change. Their parietal capsular epithelium was replaced by a layer of tall columnar cells with oval, hyperchromatic nuclei arranged with their long axes perpendicular to the basement membrane. This peculiar epithelium was generally one cell thick, but in many places, especially near the exit of the proximal convoluted tubules, it was two or even three cells in depth. Occasionally, small plications projected into the capsular space. The cells were quite uniform in size and in shape, but as they approached the reflection onto the glomerular tuft they gradually became less columnar, and the glomerular tufts were covered by the usual flattened squamous "visceral" epithelium. No mitotic figures were seen and nowhere did these proliferating cells invade beyond the basement membrane into the surrounding parenchyma. There was, however, an occasional extension, for a short distance only, into some of the proximal convoluted tubules. Spherical bodies, staining red with the Laidlaw stain for inclusion bodies, were found within the nuclei of a few of the proliferating capsular cells. They were also demonstrated within the nuclei of the proximal convoluted tubular epithelium, but since similar bodies could occasionally be found in control material their significance is questionable. The capsular spaces were generally clear, but in some glomeruli there were curious hyaline, refractile bodies, some round, others oval, and about many of them infolded hyperchromatic capsular cells were arranged in the form of rosettes. In tissue fixed with

Zenker's fluid these hyaline glomerular bodies were eosinophilic with hematoxylin and eosin, but purple-black with the azan carmine-Wilder stain. In all formalin-fixed tissue they were purple-black. The von Kossa stain revealed that they were not composed of calcium salts, and with the Laidlaw inclusion-body stain they were a pale brown. The glomerular tufts and their associated visceral epithelial cells were occasionally compressed and relatively bloodless. In the few glomeruli that appeared to escape this change it was usually possible to detect a few cells in the capsule that were thickened and hyperchromatic. Other portions of the nephron appeared normal and the only vascular abnormality was minimal arteriolar sclerosis. In the interstitial tissue of the medulla and in the adipose tissue of the pelvis a moderate infiltration with hematopoietic tissue was found.

The gallbladder showed changes consistent with the diagnosis of chronic cholecystitis and at one point its mucosa was replaced by neoplastic tissue. The neoplasm here, as well as elsewhere, was a poorly differentiated, largely necrotic adenocarcinoma, not producing mucin. Metastases were found in the liver, lungs, and regional lymph nodes. None were found in the kidneys. Although no metastases were found in the lumbar vertebrae or sternum and although the patient did not have a profound anemia, intensive extramedullary hematopoiesis was found in the spleen, liver, adrenal, stomach, as well as the kidneys.

#### COMMENT

The significance of the described alteration in Bowman's capsule is obscure. It appears to be a diffuse neoplastic change, probably primary in the kidney. A renal primary is indicated by two features. One, the cytology of the abnormal capsular epithelium differs from that of the biliary tract tumor; and two, no route for metastasis to the space of Bowman is apparent since no tumor cells are found within the glomerular tufts.

Changes roughly resembling those herein described have been noted by others in kidneys which were the sites of other primary or secondary tumors. Thus Lauterburg's<sup>1</sup> report contains a sketch of a glomerulus superficially much like that just described, but differing in two respects. First, the tumor cells lining the capsule were identical cytologically with those of a primary bronchogenic carcinoma which had metastasized extensively to the kidney. Second, only few glomeruli were involved, and the author believed that the abnormal cells were derived from localized metastatic tumor nodules, possibly extending along tubules which acted as conduits. In a peculiar case of multicentric primary carcinoma of the kidney reported by Lisa,<sup>2</sup> a single

glomerulus in a single section showed similar replacement of the capsular epithelium by a layer of tumor cells, and in addition a tumor cell was noted in the glomerular basement membrane. The latter finding together with the restriction of the change to a single glomerulus clearly distinguishes it from the changes just described. The resemblance of the diffuse capsular epithelial transformation to pulmonary adenomatosis of men and sheep (jaagsiekte)<sup>3</sup> is rather striking, but of obscure significance. Extension of proximal convoluted tubular conical cells along the glomerular parietal basement membrane has been noted in human kidneys, and is of frequent occurrence in mice,<sup>4</sup> but its resemblance to the lesion here described is too remote to suggest that the latter change is of the same nature.

If then, as appears likely, the capsular transformation found in this case represents a primary neoplastic change, the possibility that it follows the urinary excretion of some growth-inciting agent, virus, or chemical may be raised. Unfortunately, there seems, at present, no way of obtaining further information as to the cause of this unusual lesion.

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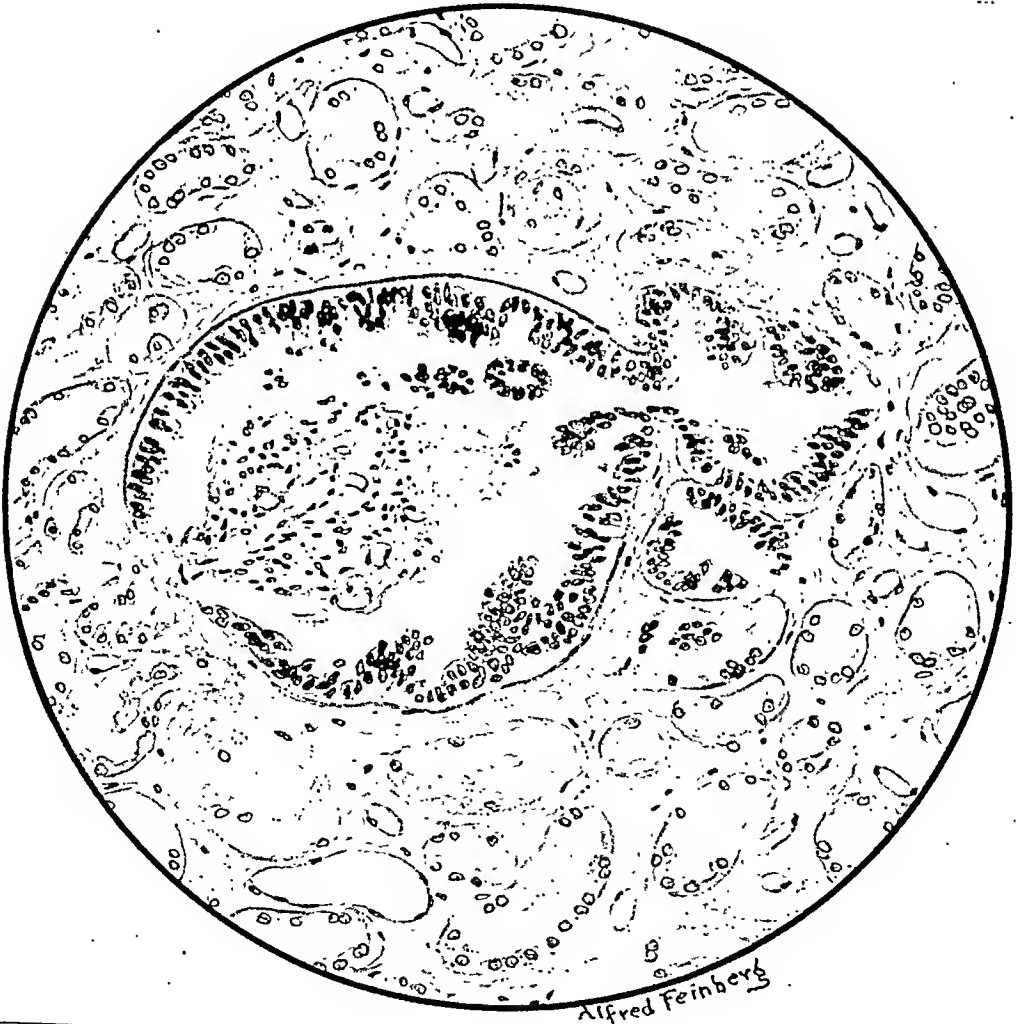
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#### DESCRIPTION OF PLATE

##### PLATE 123

FIG. 1. Kidney, showing adenomatoid transformation of capsular epithelium with occasional extension into proximal convoluted tubules. Hematoxylin and eosin stain.  $\times 95$ .

FIG. 2. Drawing of a glomerulus illustrating the chief points of the lesion. Of note are the plications, rosette formation, absence of invasion, sparing of glomerular tuft, and extension into proximal convoluted tubule. Hematoxylin and eosin stain.  $\times 215$ .



Alfred Feinberg

Glomerular Capsular Epithelium



## BISMUTH PIGMENTATION ITS HISTOCHEMICAL IDENTIFICATION \*

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On occasion it is desirable to differentiate the pigmentation produced by compounds of bismuth from those due to other causes. Pigmentary deposits containing bismuth have been described as a diffuse, dark discoloration of the colon, beginning sharply at the ileocecal valve and involving the mucosa in varying degrees.<sup>1-7</sup> Bismuth is one of the several metals which may be deposited in the gingiva or other areas of the oral mucosa and induce brown, bluish, or black discoloration.<sup>8,9</sup> Deposition of black bismuth sulfide has also been described in the vagina<sup>7,10,11</sup> and in the bladder.<sup>12,13</sup> Deep pigmentation of the skin due to bismuth deposition may occur in exceptionally rare cases.<sup>4,14,15</sup>

In the present investigation, Castel's method<sup>16</sup> for the histochemical identification of bismuth was adapted for the demonstration of bismuth sulfide in tissue sections and in gross specimens. Thus, a simple histochemical procedure for the identification of bismuth, deposited as black sulfide as it may occur in surgical or autopsy material, is available. It was found that bismuth pigmentation of the large intestines occurs fairly frequently. Among the last 340 autopsies performed at the Mount Sinai Hospital, 4 examples were encountered.

### METHOD

The method depends on the property of hydrogen peroxide of decolorizing bismuth sulfide instantaneously. Black bismuth sulfide is thus transformed into white bismuth sulfate.<sup>17</sup> By then treating the sections with a slightly modified Castel reagent,<sup>16</sup> containing brucine sulfate and potassium iodide, the bismuth sulfate is transformed into an orange-red deposit. This reaction, based on the work of Léger,<sup>18</sup> depends on the fact that numerous organic bases form insoluble double iodides with bismuth of the general formula  $BI_3 \cdot B \cdot HI$ , in which B represents the base.<sup>19</sup>

In this study, frozen as well as paraffin sections were used. Paraffin sections were deparaffinized in the usual manner and were then treated with a few drops of superoxol (30 per cent hydrogen peroxide, Merck). This reagent is best kept in a dark bottle in the refrigerator. The black color of bismuth sulfide disappears in a few seconds. Sections

\* Received for publication, June 11, 1945.

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are then washed thoroughly in tap water and placed in a Coplin jar, containing the modified Castel reagent.<sup>16</sup> The latter is made by dissolving 0.25 gm. of brucine sulfate (Merck or Eastman Kodak) in 100 cc. of distilled water containing 2 or 3 drops of concentrated sulfuric acid. After the brucine sulfate has dissolved, 2 gm. of potassium iodide are added. The reagent is kept in a brown bottle and filtered before use. After 1 hour, the sections are put into another jar containing the brucine reagent diluted with three parts of distilled water, and gently shaken in order to remove precipitates. Most of the remaining fluid is removed by gentle blotting and the slide covered with levulose solution (prepared by dissolving 30 gm. of levulose in 20 cc. of water by heating to 37° C. for 24 hours), to which a drop of the diluted Castel reagent is added with a glass rod or toothpick. If a counterstain is desired, the slide is stained for 4 minutes with a mixture containing 100 cc. of the nondiluted brucine sulfate reagent and 1 cc. of a 1 per cent aqueous solution of light green SF (Hartman-Leddon Co.), filtered before use. The stain will keep well although the orange color may darken to some degree.

This method can be applied also to gross specimens. Fixed material is preferable, but fresh tissues are also suitable. The concentrated hydrogen peroxide solution is added drop by drop to the area under investigation. Decolorization will take place immediately if bismuth sulfide is present. The specimen is then thoroughly washed in running water for several minutes to remove excess hydrogen peroxide. The modified Castel reagent is then applied to this surface. An intense orange precipitate is formed, giving the same color to the mucosa.

## CASES WITH BISMUTH PIGMENTATION

### Case 1

A white male, 41 years old, was admitted to the hospital because of dyspnea, generalized edema, and severe anemia. Physical examination revealed marked enlargement of heart, liver, and spleen. Petechiae were found on the conjunctivae. A positive Wassermann reaction of the blood was present. For this reason the patient was given biweekly bismuth injections. He underwent a splenectomy for intractable anemia with good results, and was discharged after 4 months. Following this he continued to receive weekly bismuth injections. Five months later he was readmitted in cardiac failure, and died after 14 days in the hospital.

The pertinent post-mortem findings (autopsy No. 12768) were cerebral hemorrhage, healing stage of subacute bacterial endocarditis upon chronic rheumatic mitral and aortic valvular disease, and subacute glomerulonephritis. Patchy gray and black areas of discoloration were found in the cecum. In the tonsils similar pigmentation was visible in the crypts. Microscopic sections through the discolored areas

of the cecum showed black pigment in the walls of the most superficial capillaries. Characteristically, this pigment was found in the capillary endothelium, producing a singly or doubly contoured black line. Very little pigment was seen elsewhere. Sections through the tonsils showed lymphatic tissue covered by one layer of cylindrical epithelium frequently arranged in a papillary fashion. The subepithelial capillary walls showed marked impregnation by a dark pigment (Fig. 1). The intensity of this reaction varied in different vessels, so much so that in some the lumen was obscured by the very heavy deposit. Moreover, black pigment was seen also in some histiocytes as well as free in the submucosa, without definite relation to cellular structures.

### *Case 2*

A white female, 30 years old, had a history of rheumatic fever and chorea in childhood. She had been suffering from mild dyspnea for the past 5 years. Two years before admission hypertension was noted. Wassermann reaction of the blood was 4 plus and therefore she was treated elsewhere with weekly bismuth injections for 1 year. Because of sudden severe dyspnea and cyanosis she was admitted to the Mount Sinai Hospital, where her blood pressure was found to be 250/140 mm. Hg. Urine examination revealed 3 plus albumin, hyaline and granular casts, and occasional red blood cells. She died 2 days later.

The pertinent post-mortem findings (autopsy No. 12933) were rheumatic heart disease and malignant nephrosclerosis. The cecum and ascending colon showed a very marked, diffuse, black discoloration. On microscopic study, the findings in regard to the location of pigment were exactly as in the previous case. Pigment-laden histiocytes and extracellular granules in the mucosa were, however, quite prominent. Again, no pigment was seen in the deeper layers (Fig. 2).

### *Case 3*

A white male, 65 years old, gave a history of chancre 25 years before admission to the hospital. Eight months before, his blood and spinal fluid had given a positive Wassermann reaction and a paretic colloidal gold curve. He was then treated with fever therapy, arsphenamine, and two injections of 1 cc. of a bismuth preparation in oil. Five weeks before admission, a gastrostomy was performed elsewhere because of carcinoma of the esophagus. The patient entered Mount Sinai Hospital for resection, but died within a few days and before operation.

The most important post-mortem findings (autopsy No. 12952) were squamous cell carcinoma of the esophagus with metastases to the lungs and lymph nodes, anthracosilicosis, pulmonary tuberculosis with recent bronchogenic spread, and syphilitic mesaortitis. The mucosa of the cecum and ascending colon was diffusely brown. Microscopic sections through this area showed only occasional superficial capillaries impregnated with a dark pigment. Fine pigment granules were seen in the endothelial cells of less involved capillaries, and in an occasional histiocyte in the mucosa.

*Case 4*

A white male, 55 years old, had a history of chancre 34 years prior to admission and of treatment for 7 years with injections of bismuth and arsphenamine. Three years before admission examination of his blood revealed a 1 plus Wassermann reaction. He was treated for another year with bismuth and arsphenamine. Thereafter, his serologic tests became normal. A few months later he acquired a second syphilitic infection, proved by dark-field examination. Again he was treated with bismuth and arsphenamine parenterally. The patient was admitted to the hospital because of vomiting, severe headache, and mental confusion. His blood pressure was 250/110 mg. Hg and blood urea nitrogen, 150 mg. per cent. The patient died 3 days later.

The significant findings at the post-mortem examination (autopsy No. 13022) were malignant nephrosclerosis with marked cardiac hypertrophy and dilatation. The large intestine showed diffuse black pigmentation of the mucosa, especially severe in the cecum, starting sharply at the ileocecal valve and diminishing in intensity distally. A pigment line was visible on the upper gingival margins. Microscopic sections of the colon showed, as before, impregnation of many of the capillary walls by black pigment, and altogether a similar histologic picture. Sections through the gingiva revealed pigment deposited in superficial capillaries and histiocytes, as well as severe chronic and acute inflammation with superficial ulceration.

*Case 5*

A white male, 59 years old, had had a generalized rash, including palms and soles, at the age of 26. He was treated with mercury inunctions. Thirteen years later examination of his blood revealed a 4 plus Wassermann reaction. His spinal fluid was negative at that time. For the following 3 years he was treated continuously with bismuth and salvarsan injections. Examination of his blood 2 years prior to admission revealed a positive Wassermann reaction. He was given a course of eighteen bismuth and arsphenamine injections, following which the Wassermann reaction of the blood became negative. On one occasion, when the patient visited the Out Patient Department complaining of a swelling in his mouth, physical examination revealed a small growth extruding between the third and fourth left lower teeth. No mention was made of a black discoloration of the gingiva.

A specimen of this area, taken for biopsy (surgical specimen No. 81006), showed marked acute and chronic inflammation. Except for a greater intensity, the gingiva here revealed pigment deposition similar to that seen in the preceding case.

## COMMENT

Microscopically, the outstanding feature of the deposition of bismuth is the impregnation of the capillary walls, predominantly in the superficial layers of the mucosa. When little is present, it is found only here; when much pigment is deposited, bismuth sulfide is seen also in histiocytes and free in tissue spaces. It is never encountered

in the deeper layers. This behavior is identical in the mucous membrane of the mouth and in that of the large intestine. In all cases in which bismuth sulfide was seen, it could be identified easily with the above-described method.

Among the pigments occurring in the oral cavity, melanin and iron are easily distinguished by their location. Iron, which may be present as the black sulfide, is oxidized by concentrated hydrogen peroxide and transformed into the usual golden brown hemosiderin. It does not react with Castel's reagent<sup>16</sup> but gives the typical iron reactions. Melanin is not bleached by a short treatment with hydrogen peroxide and does not give a reaction with the brucine reagent. Among the exogenous pigments, lead sulfide is the most important to exclude. Since it is deposited in vessel walls in the same manner as bismuth, it cannot be differentiated in routine sections. In order to test the bismuth reagent on lead sulfide in tissue sections, slides prepared according to Gomori's method<sup>20</sup> for the demonstration of acid phosphatase were used. By this technic, the phosphatase activity is demonstrated by the deposition of black lead sulfide. Preparations of this kind were oxidized with hydrogen peroxide. The lead sulfide is immediately discolored in the same fashion as bismuth sulfide, the sulfate being formed. However, treatment with Castel's reagent brings about only a slightly yellowish tinge of the lead deposits ( $\text{PbI}_2$ ), in contrast to the brilliant orange-red formed with bismuth.

Copper, silver, and mercury may also be deposited in capillary walls. No tissue material was available for study. However, in the test tube, as already shown by Castel,<sup>16</sup> silver and mercury give yellow, and copper gives brown precipitates, thus differing from bismuth. All these metals react with the iodide but do not combine with the brucine to form double salts as bismuth does.<sup>19</sup>

In the large intestine, the most frequent cause of dark pigmentation is "melanosis." In this condition, a brown granular pigment is deposited in the histiocytes of the lamina propria and occasionally in the submucosa. This pigment has been grouped with the melanins.<sup>21, 22</sup> It is usually found in persons suffering from constipation. It has been shown that its occurrence is dependent upon the intake of the emodin-bearing group of cathartics (cascara, aloes, frangula, Rheum, and senna).<sup>23, 24</sup> On microscopic examination, a distinction between bismuth and this pigment is readily made, since the latter never impregnates the capillary walls. An immediate distinction can be made at autopsy by applying the previously described gross reaction to fresh tissue. This melanin is not discolored by the short application of hydrogen peroxide and does not give the reaction with Castel's reagent.<sup>16</sup>

A second specific test, also based on Léger's work,<sup>18</sup> is available for identification of bismuth in tissue sections. Quinine sulfate and potassium iodide give a yellow precipitate with bismuth. Komaya,<sup>25</sup> among others,<sup>4</sup> adapted this reaction for the use in tissue sections. While this method gives good results in frozen sections, we found Castel's reagent<sup>16</sup> much more reliable and simpler for use in paraffin-embedded material.

No attempt was made to apply the specific bismuth stain systematically to all organs. However, on applying the stain to the kidney sections of cases 2 and 4, as well as of another case not included in this series, it showed refractile globules in the epithelium of the proximal convoluted tubules, as described by Pappenheimer and Maechling.<sup>26</sup> These authors studied the staining properties of these inclusion bodies extensively. They found them to react regularly with the Weigert-Spielmeyer stain. They became dark on treatment with hydrogen or ammonium sulfide, but did not give the more specific histochemical reactions for bismuth with stannous chloride-sodium hydroxide and with Komaya's reagent.<sup>25</sup> Apparently, similar bodies were described also by Langhans<sup>27</sup> in tissue sections of experimental animals, and in epithelial cells of human urinary sediments by others.<sup>28-32</sup> In our material most of these inclusion bodies reacted with Castel's reagent.<sup>16</sup> The majority gave a distinct orange color, although some stained yellow and others remained unstained. Desquamated epithelial cells and casts gave an occasional positive reaction.

To our knowledge no statistics are available concerning the incidence of bismuth pigmentation in the colon. Wiener<sup>11</sup> and Heyman<sup>7</sup> stated that deposition of bismuth is of very rare occurrence. However, since special attention has been paid to proper identification of discolored areas in the mucosa of the large intestine, we have encountered this condition more frequently.

Case 3 is of special interest. Here bismuth pigmentation of the colon was found although only two injections of 1 cc. of a bismuth preparation were given. Grossly, "melanosis coli" was diagnosed. However, the pigment showed the typical microscopic appearance and histochemical behavior of bismuth sulfide. Because of this, inquiries were made which revealed that the patient had received this small amount of bismuth at another hospital. The amount of histologically demonstrable bismuth varied markedly in the other three patients, although all of them had received intensive treatment. This is in full agreement with the findings of Sollmann, Cole, and Henderson<sup>33</sup> who determined the bismuth content of various organs of patients who had received bismuth. In 23 cases the colon contained between 0.025 and

3.0 mg. of bismuth in 100 gm. of wet tissue. The average was 0.115 mg. per 100 gm. of wet tissue. They found that the colon ranked fifth in bismuth content, after kidney, liver, spleen, and bile. In one case, described previously by one of us,<sup>6</sup> in which bismuth therapy led to fatal intoxication, 5.8 mg. of bismuth per 100 gm. of wet tissue was found in the colon.

### SUMMARY

Castel's method for the demonstration of bismuth in tissue preparations was adapted for the identification of bismuth sulfide in frozen sections, paraffin sections, and in gross specimens. The method permits histochemical identification of bismuth sulfide pigmentation in any tissue. Bismuth discoloration of the colon is apparently not infrequent. It was found in four of 340 consecutive autopsies. Even small amounts of injected bismuth may lead to the deposition of histochemically demonstrable bismuth sulfide in the large bowel. The inclusion bodies found in the renal epithelial cells following the use of bismuth preparations frequently give a positive reaction with Castel's reagent.

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## DESCRIPTION OF PLATE

### PLATE 124

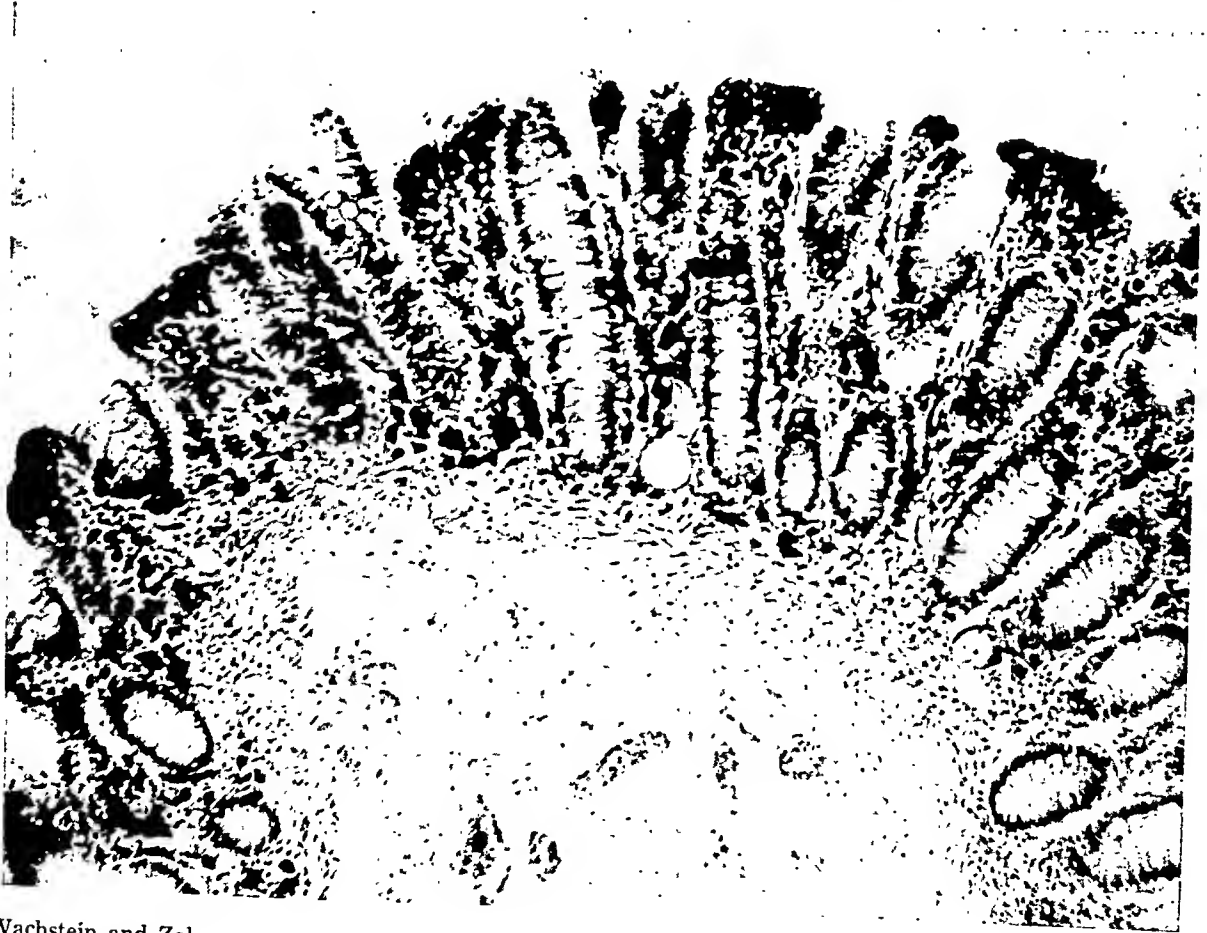
FIG. 1. Case 1. Section through tonsil, showing superficial capillaries impregnated by black bismuth sulfide. Occasionally pigment is seen in histiocytes. Hematoxylin and eosin stain.  $\times 145$ .

FIG. 2. Case 2. Section through colon, showing extensive deposition of bismuth sulfide in mucosa. Hematoxylin and eosin stain.  $\times 70$ .

1



2



Wachstein and Zak

Histochemical Identification of Bismuth





## OSSIFYING CARTILAGE AND THROMBI IN THE HEARTS OF RATS \*

EDMOND J. FARRIS, Ph.D., ELEANOR H. YEAKEL, Ph.D., and MARGARET M. SEITNER, M.A.  
(From *The Wistar Institute of Anatomy and Biology, Philadelphia 4, Pa.*)

Dissection and autopsies of rats in The Wistar Institute animal colony have disclosed two types of grossly visible pathologic lesions in the heart; namely, thrombi in the left atrium and ossifying cartilage in the left ventricle. Of gray Norway and Wistar albino rats 336 and 807 were examined, respectively, these rats being part of a colony established for studies in aging. A number were dissected after anesthetization to provide material for research on senescence, and the remainder were autopsied after dying from natural causes.

### THROMBOSIS

Thrombi were detected in the left atrium only. They were seen in rats of both strains after death from disease, and in gray Norways after anesthetization, when agonal thrombosis seemed unlikely. All of the thrombi were pale yellow, firm, and in many instances large enough to fill the chamber completely. Usually each was attached loosely to the atrial wall. Microscopic preparations of thrombi from 2 autopsied rats showed no signs of organization, resembling in this respect the thrombi described by Wilens and Sproul.<sup>1</sup> These authors remarked that fairly old rats (over 700 days of age) were particularly susceptible to the lesions. Although the average age of rats with cardiac thrombi autopsied by us was 773 days, thrombi were seen in gray rats dissected at 200 days (3 animals) and 400 days (2 rats) of age, and in an autopsied animal aged 229 days. The lesions were observed in a total of 24 gray Norway rats and 2 albinos, comprising 7 per cent of all grays examined, and considerably less than 1 per cent of all albinos.

### OSSIFYING CARTILAGE

Routinely the left ventricle was opened by a longitudinal incision, and the blood removed. Occasionally there was disclosed an ivory-colored, brittle structure clinging to the surface of the wall, varying in shape from a single roughened spicule to a coarse network with irregular branchings. Each structure was attached loosely to the endocardium and could be pulled away intact when sufficient traction was exerted upon it. The usual site was at or near the apex.

Two hearts containing prominent spicules were cleared and treated with alizarin-red-S. In both specimens the spicules stained a bright

\* Aided in part by a grant from the Samuel S. Fels Fund.

Received for publication, June 22, 1945.

red, indicating the presence of calcium. Figure 1 is an enlarged photograph of the cleared hearts, showing the gross structure of the calcified bodies.

Microscopic sections were made of 7 specimens, *in situ*. In most instances the structures were composed of cells resembling the hypertrophied or senescent cartilage cells found in endochondral bone formation, imbedded in a coarse-fibered matrix. A typical section is shown in Figure 2, in which part of the ground substance is seen to be calcified. Cavities surrounded by the cartilage-like tissue, and containing reticular cells and capillaries suggestive of primitive marrow, were frequently observed. Examples are shown in Figures 3, 4, and 5. Evidence of partial ossification may be discerned in Figure 3. One lesion was found in which the tissue resembled a spicule of heterotopic bone or osteoid tissue, with small cells lying in a compact, homogeneous matrix (Fig. 4). Elsewhere in the same heart another lesion was composed entirely of hypertrophied cartilage-like cells, as in Figure 2.

At their points of attachment to the heart wall, the cartilaginous structures lay beneath the endocardium in fibrous tissue continuous with the heart muscle (Fig. 5). A sheath of tissue continuous with the endocardium surrounded the remainder of the spicule that lay free in the lumen of the ventricle.

Grossly, the calcified structures were seen only in the left ventricle. Microscopic study of one heart revealed cartilage-like tissue imbedded within the wall of the left atrium (Fig. 6). The appearance of the surrounding area suggested early formation of cartilaginous tissue. Adjacent to this, the heart wall showed evidence of chronic inflammatory changes.

Calcified bodies were observed in the hearts of 7 per cent (25 rats) of the gray Norways examined, and in less than 1 per cent (7 rats) of the albinos. The discovery of some heterotopic tissue only by microscopic examination makes a more accurate estimate of its frequency impossible until a large series of preserved hearts is studied. However, on the basis of the data at hand, gray rats appear to be more susceptible to this pathologic condition, as they were to thrombosis. Moreover, the spicules in their hearts were, for the most part, decidedly larger than those in the albinos, and were found at an earlier age, the youngest gray rat being 364 days old, while the youngest albino with visible spicules was 830 days of age. The oldest gray Norway with the lesion was dissected after anesthetization at 1055 days of age; the average age for the group was 734 days. The average age of the albinos with heart spicules was 880 days. During the life of these rats no symptoms were noticed that indicated impaired efficiency of the heart.

## DISCUSSION

Calcium deposits of an amorphous appearance have been observed in the hearts of rats,<sup>2</sup> and calcification of the aortic ring, with cartilage and/or bone formation, has been reported in various animals, including rats.<sup>3</sup> In the experimental production of bone in the aortas of rabbits, Harvey<sup>4</sup> found chiefly osteoid tissue, but occasionally an intermediate tissue that resembled cartilage. This process, occurring usually in an area of calcareous degeneration, he considered to be one of metaplasia of the connective tissues. It is thought that the ossifying cartilage and bone described by us arose from fibroblasts in areas of degeneration or scarring of the heart, the cause of which is unknown. If the condition here reported is analogous to Harvey's results, it would appear that in the rats of our colony the predominant metaplastic tissue in the heart is cartilaginous in type. The fibroblasts apparently metamorphosed into large, swollen cells resembling senescent cartilage cells, frequently within a calcified matrix, but stopped short, for the most part, of transformation into tissue more nearly resembling bone.

## SUMMARY

The hearts of 336 gray Norway rats and 807 Wistar albinos were examined grossly for abnormalities.

Thrombi were found in the left atria of 24 gray Norways and 2 albinos.

Calcified structures, loosely attached to the chamber wall, were seen within the left ventricles of 25 gray rats (including 11 with thrombi in the atrium) and 7 albinos (including 1 with a thrombus).

Microscopically, the calcified structures were composed chiefly of large cells resembling the senescent cartilage cells of endochondral bone formation, although areas resembling osteoid tissue were occasionally seen. The matrix was frequently calcified, and tissue resembling primitive marrow was often present. At the points of attachment to the heart wall, the heterotopic tissue was imbedded in fibrous connective tissue.

It is assumed that the ossifying cartilage-like tissue arose from the metaplasia of fibroblasts in areas of degeneration or scarring, the cause of which is unknown.

We wish to express our appreciation to Dr. Robert C. Horn, Jr., of the Hospital of the University of Pennsylvania, for examination of the microscopic sections and advice.

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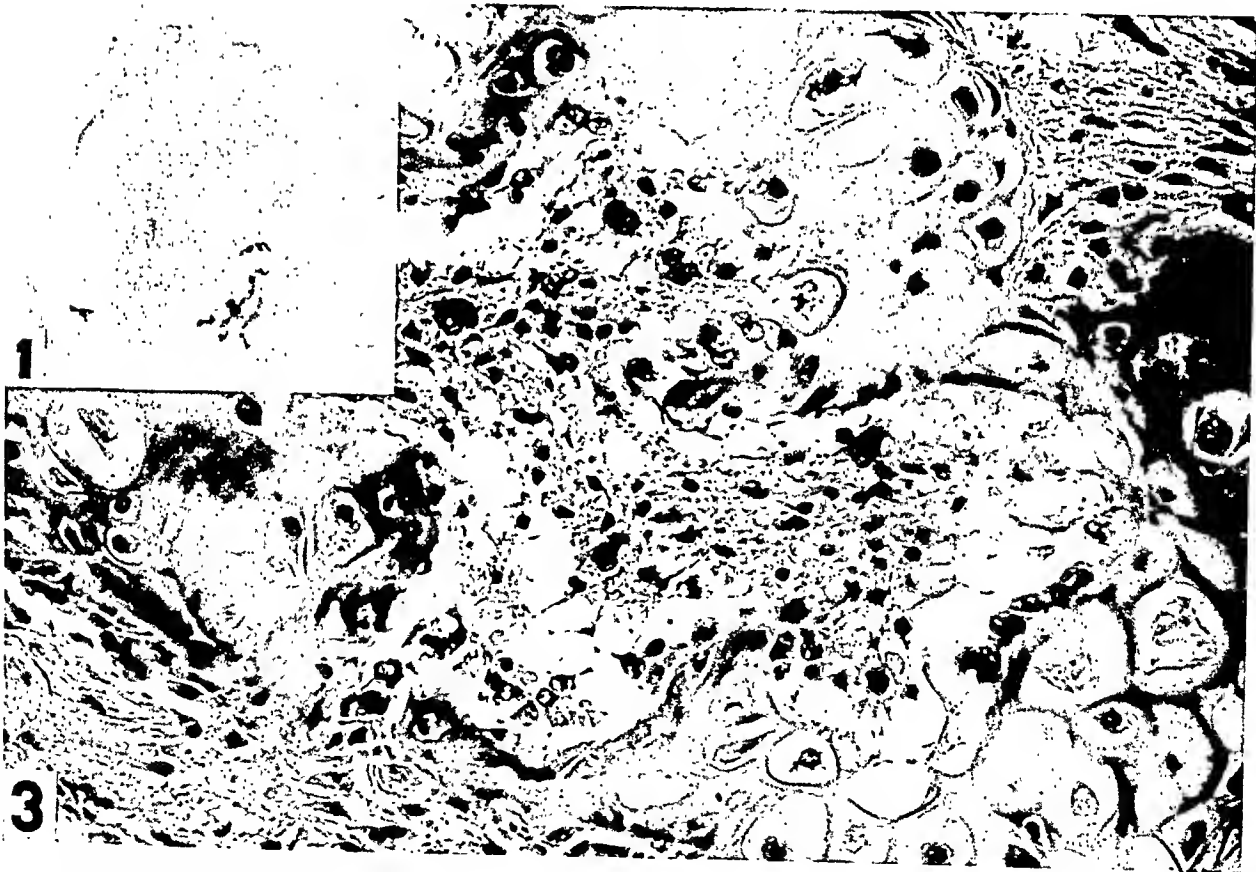
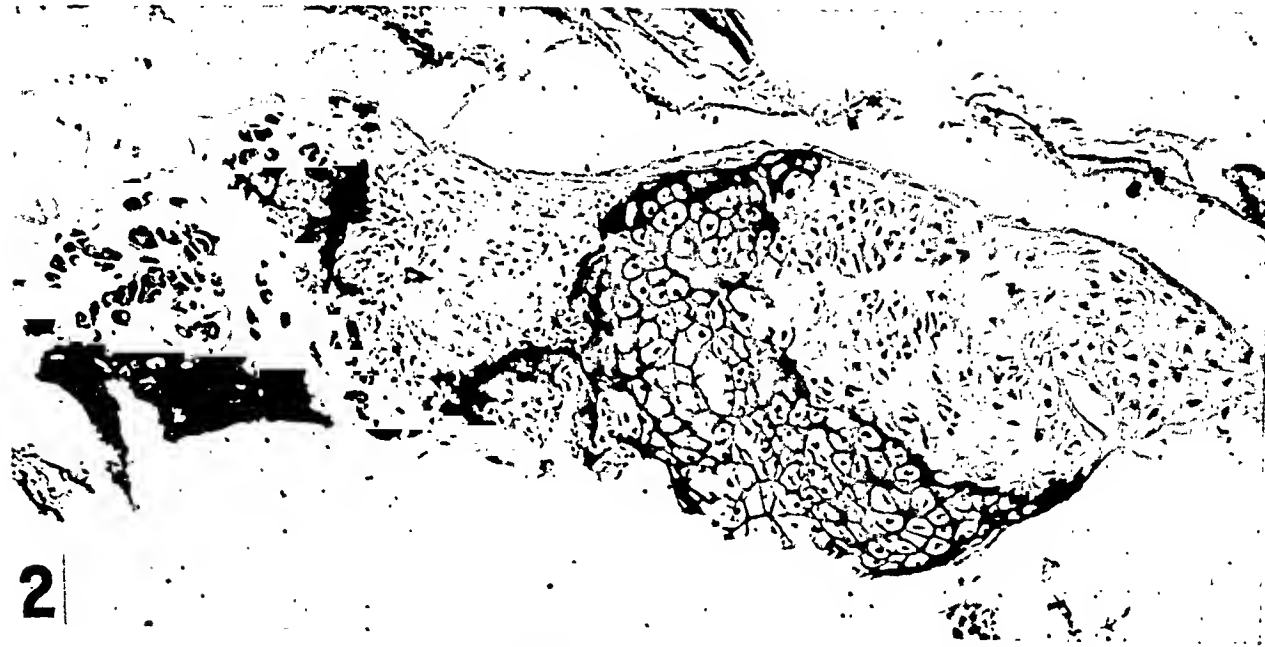
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## DESCRIPTION OF PLATES

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### PLATE 125

- FIG. 1. Heart of rat cleared with KOH and stained with alizarin-red-S. Calcified bodies are visible in the left ventricle, which was laid open.
- FIG. 2. Spicule lying in the lumen of the left ventricle. Calcification and dispersal of cartilage-like cells are shown.  $\times 125$ .
- FIG. 3. Marrow-like cavity within a spicule, with apparent erythropoiesis.  $\times 430$ .



Farris, Yeakel, and Seitner

Cartilage in the Hearts of Rats

PLATE 126

FIG. 4. Bone surrounding marrow cavity. Capillaries are visible.  $\times 395$ .

FIG. 5. Attachment of cartilaginous structure to ventricle wall.  $\times 115$ .

FIG. 6. Cartilaginous tissue imbedded in atrial wall.  $\times 115$ .







# ANOMALOUS PORTAL VEIN IN MICE OCCASIONALLY CAUSING INTESTINAL INFARCTION \*

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New York 21, N.Y.)

An anomaly of the portal vein has frequently been observed in mice of two inbred strains, and this has often proved fatal by causing hemorrhage, infarction, or obstruction of the intestine. A similar abnormality has been encountered in man.<sup>1</sup>

Normally the portal vein in mice, as in rats,<sup>2</sup> receives the splenic, superior mesenteric, and pyloric veins and then courses dorsal to the duodenum, with the hepatic artery and common bile duct, to the liver. The portal vein may, however, pass ventrally across the duodenum to the liver and remain unattached except distally where it leaves the mesentery, and proximally at its entry to the liver (Fig. 1). The hepatic artery and common bile duct are then separated from the anomalous portal vein and follow their usual path. No associated abnormalities of other blood vessels or of viscera have been observed.

In most of the observed cases this variation in the course of the portal vein had caused no signs and was only an incidental finding at autopsy. In a number of instances, however, loops of the small intestine had herniated through the space behind the vein, causing obstruction by elongating and compressing the vein, with resulting hemorrhagic infarction of the mesentery, intestine, and spleen (Figs. 2 and 3). In a few cases, the vein appeared to have strangulated the intestine, causing distention of the intestine and stomach above the point of constriction. Perforation and peritonitis were not seen.

This abnormality has thus far been observed at autopsy in more than 380 mice, in 24 of which intestinal infarction or obstruction, as described above, caused death. The malposition occurred most frequently in mice of the Ak stock, a high-leukemia stock of mice which has been inbred in this laboratory by brother-sister matings for more than 30 generations. The total incidence of the anomaly in the Ak stock has been 26 per cent. Table I shows that in three sublines of the Ak stock the frequency varied, but that in each subline it occurred approximately twice as often in female as in male mice. These figures are undoubtedly lower than the actual numbers since the condition may be difficult to recognize at autopsy, especially if post-mor-

\* These observations were made in the course of studies on leukemia, carried out with support from The Lady Tata Memorial Trust, The Jane Coffin Childs Memorial Fund for Medical Research, and The International Cancer Research Foundation.

Received for publication, May 17, 1945.

tem decomposition has become advanced or if the vein is empty and appears as a mere fibrous strand between the liver and the mesentery. Two per cent of mice of the Rf stock, which is a highly inbred, low-leukemia stock, have also been found to have this abnormality. It has appeared in various hybrid combinations of the Ak mice with other

TABLE I

*Incidence of Anomalous Position of Portal Vein in Ak and Rf Inbred Strains of Mice*

Stock of mice	Females			Males		
	Number observed	With anomaly		Number observed	With anomaly	
		Number	Per cent		Number	Per cent
Subline Akl	137	61	45	90	22	24
Subline Akn	161	53	33	113	15	13
Subline Ako	339	93	27	248	34	14
Total Ak	637	207	32	451	71	16
Total Rf	130	4	3	78	0	0

stocks; the incidence appears to be lower in these than in the Ak mice, but the data are insufficient for analysis.

In an attempt to determine a genetic basis for this congenital variation, two litters of Ak mice in which the course of the portal vein had been ascertained by laparotomy were bred in various combinations, as shown in Table II. Of the 7 mice in each of these litters, 2 in one and

TABLE II

*Incidence of Anomalous Position of Portal Vein in Offspring from Selected Ak Mice in Which the Course of the Portal Vein Was Known*

Parents	Offspring					
	Females		Males		Total	
	Produced	With anomaly	Produced	With anomaly	Produced	With anomaly
♀ normal × ♂ normal	6	3	6	1	12	4
♀ normal × ♂ with anomaly	8	4	14	3	22	7
♀ with anomaly × ♂ normal	15	8	9	2	24	10
♀ with anomaly × ♂ with anomaly	5	1	16	5	21	6
Total	34	16	45	11	79	27

5 in the other had the abnormality. Offspring with and without the anomaly were produced by each of the mating combinations, with no significant differences in the proportion obtained from the various matings; and the total incidence in offspring from all of these matings agreed closely with that obtained by unselected breeding of the Ak stock. Despite the slightly different percentages of mice with this venous malposition in the various sublimes of the Ak stock, no tend-

ency was observed for families within each line to have a high or a low incidence of this condition.

Thus a genetic basis for this anomaly has not been definitely established. The absence of significant differences among the progeny from various mating combinations is similar to findings on the incidence of leukemia in various mating combinations of the Ak stock. Spontaneous leukemia has been observed in approximately 77 per cent of the females and in 61 per cent of the males of the Ak mice. The figures vary slightly among sublimes of the stock; however, in each subline the incidence in the progeny is the same, whether both, one, or neither of the parents develop leukemia.<sup>3</sup> It is noteworthy that both leukemia and this anomaly occur more frequently in female mice than in males of the Ak stock, and both are infrequent in mice of the Rf stock. There has been, however, no correlation between the incidence of leukemia and that of this venous abnormality. In the case of leukemia, these findings are interpreted by assuming that the incidence of leukemia as determined by hereditary factors is to some extent controlled by extrinsic factors.<sup>4</sup> Such an explanation does not seem adequate for a congenital anomaly.

Anomalies of the portal vein in man are frequent in association with partial or complete situs inversus of the abdominal viscera, though rare in its absence.<sup>1</sup> Pernkopf<sup>1</sup> has reported a case in man in which the portal vein was in the preduodenal position but was not accompanied by situs inversus, and has discussed numerous variations in the embryological development of the portal vein. This anomaly results from variations in the development of the portal vein from the peri-intestinal rings formed by the vitelline veins in the embryo. Anomalies of the intra-embryonic portion of the vitelline vein in a pig embryo and a human embryo have actually been noted by Begg.<sup>5</sup>

The observation<sup>6</sup> that various congenital defects in infants are produced by infection of the mother with rubella during the early months of pregnancy suggests the possibility that instead of, or in addition to, a genetic factor, some mild or latent infection might be responsible for the occurrence of the anomalous course of the portal vein in mice.

#### SUMMARY

An anomalous preduodenal position of the portal vein, not associated with other vascular anomalies or variations in the position of the abdominal viscera, has been observed in 380 mice. In 24 mice it caused death by producing intestinal obstruction or infarction. A similar anomaly has previously been described in man.

Most of the cases occurred in mice of the highly inbred stock Ak,

in which the incidence of the anomaly was 26 per cent. It has also been observed in 2 per cent of mice of another highly inbred stock (Rf) and in hybrids of the Ak stock. The incidence of the anomaly in female mice was approximately twice that in males.

Attempts to determine a genetic basis for this venous malposition by breeding of selected Ak mice were unsuccessful.

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#### DESCRIPTION OF PLATE

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##### PLATE 127

FIG. 1. A mouse in which the portal vein is ventral to the duodenum. The liver has been turned back to expose the vein and duodenum.

FIGS. 2 and 3. Intestinal infarction in mice, produced by herniation of loops of intestine behind the portal vein in the anomalous position. In Figure 3, the vein is the thin strand indicated by the arrow.

1



2



3



Boon

Anomalous Portal Vein in Mice



FORTY-THIRD ANNUAL MEETING  
OF THE  
AMERICAN ASSOCIATION OF PATHOLOGISTS  
AND BACTERIOLOGISTS

CHICAGO

MARCH EIGHTH AND NINTH, 1946





# THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Forty-Third Annual Meeting, University  
of Chicago, Chicago, Illinois  
March Eighth and Ninth, 1946

PRESIDENT CANNON IN THE CHAIR

## BUSINESS MEETING

March Eighth, 1946

Upon nomination of the Council, the Association voted to elect the following officers:

<i>President</i>	WILEY D. FORBUS
<i>Vice-President</i>	MALCOLM H. SOULE
<i>Secretary</i>	HOWARD T. KARSNER
<i>Treasurer</i>	ALAN R. MORITZ
<i>Incoming Member of Council</i>	TRACY B. MALLORY
<i>Assistant Secretary</i>	WILLIAM B. WARTMAN

The Secretary announced the election of Dr. Malcolm H. Soule to succeed himself as Associate Editor of *The American Journal of Pathology* for the ensuing year.

The Council announced the election of Dr. Howard T. Karsner to succeed himself on the Editorial Board of *The American Journal of Pathology*.

The Secretary announced the election of the following new members:

Margaret Bevans, New York	Elson B. Helwig, St. Louis
Richard F. Birge, Des Moines	George K. Higgins, New York
Charles M. Blumenfeld, Sacramento	Leslie S. Jolliffe, Newton Highlands, Mass.
Milton D. Bosse, Pittsburgh	John F. Kent, Washington
Henry Bunting, New Haven	Philip M. LeCompte, Brookline, Mass.
Arthur A. Case, Columbus, Ohio	Aaron E. Margulis, New York
Robert C. Dunn, Bethesda, Md.	Paul Michael, Oakland, Calif.
Stanley H. Durlacher, New Haven	Nathan Mitchell, Albany
Curtis M. Flory, New York	Robert H. More, Montreal
Richard H. Follis, Durham, N.C.	

Leo D. Moss, Olean, N. Y.  
Francis P. Parker, Atlanta  
S. B. Pessin, Milwaukee  
William R. Platt, St. Louis  
John T. Read, Columbus, Ohio  
Stanley L. Robbins, Brookline,  
Mass.

Philip Rosenblatt, New York  
Vinton D. Sneed, Portland, Ore.  
Joseph Stasney, Philadelphia  
James S. Taylor, Kingston, N.Y.  
Wilbur C. Thomas, Winston-  
Salem, N.C.  
Paul A. Van Pernis, Chicago

Onio O. Williams, Phoenix, Ariz.

The Secretary reported the reinstatement to membership of Drs. Frederic H. Foucar, Joseph C. Ehrlich, and Arthur J. Vorwald.

The Council voted to accept with regret the resignations of Drs. Stuart Graves, Calvin G. Page, and William H. Woglom.

The Council voted to record with regret the deaths of Drs. Sam S. Blackman, Jr., Newton Evans, John C. Grill, Edgar S. Ingraham, Jr., Lewis H. Koplik, and Isaac Levine.

The Secretary announced that the next meeting of the Association will be held as guests of the Jefferson Medical College, Philadelphia, Pennsylvania, on the Friday and Saturday immediately preceding the meetings of The Federation of American Societies for Experimental Biology.

The Secretary announced that the Council had voted to have a symposium in 1947 on "Necrotizing Hepatic Injury and Sequels" and appointed Lt. Col. Balduin Lucké as referee.

## SCIENTIFIC PROCEEDINGS

### THE BETA GRANULES IN THE ISLETS OF LANGERHANS IN DIABETES MELLITUS. E. T. Bell, Minneapolis, Minn.

*Abstract.* With the use of Gomori's stain the beta granules of the islets of Langerhans have been studied in 50 pancreases from nondiabetic and 30 pancreases from diabetic patients. The beta granules were clearly shown in all of the non-diabetic persons.

Of the 30 patients with diabetes, the beta granules were completely absent in 11, and present in very small quantity in 10 others. In these 21 cases the diagnosis of diabetes was made with the Gomori stain, and, since only 9 of them showed hyaline islets, there were 12 cases which could be recognized only by this stain. In 5 cases the beta granules were only moderately reduced, so that the diagnosis of diabetes was uncertain; and in 4 cases the beta granules were present in normal numbers. Two of the cases with normal granulation were clinical examples of severe diabetes. The beta granules are evidently related in some way to the formation of insulin, but their significance is not understood.

One islet cell adenoma of the pancreas, associated with severe hypoglycemia, showed only a few beta granules.

#### *Discussion*

(Dr. Israel Davidsohn, Chicago, Ill.) What about the time interval since death? Did that affect the stain?

(Dr. Bell) Post-mortem changes did not affect the islets any more than they did the acinar cells of the pancreas. Material obtained 12 hours after death shows them quite well, and in any pancreas that was not autolyzed you could see the beta granules, so we did not have to have a fresh pancreas to make this stain work.

### LYMPHADENOID GOITER. ITS DIFFERENTIATION FROM CHRONIC THYROIDITIS. C. C. Parmley (by invitation) and C. A. Hellwig, Wichita, Kan.

*Abstract.* The histologic findings in 14 lymphadenoid goiters are presented because alterations of the thyroid epithelium are, in our opinion, more important characteristics of this disease than the accumulations of lymphoid tissue. These epithelial changes have not been correctly interpreted in recent studies.

Two types of epithelial alteration are present in all of our cases: (1) small slit-like acini with high cuboidal epithelium and loss of colloid, and (2) strands of large, pale oxyphilic cells which resemble liver or adrenal cells. The latter are identical with the so-called Hürthle cells. Hürthle cell tumors occur almost exclusively in women over 40 years of age, the same as lymphadenoid goiters. These large, pale cells represent, in our opinion, an attempt to compensate for the exhaustion of colloid in the small acini.

Lymphadenoid goiter is explained best by a sequence of events similar to those of thiouracil goiter, except that the disturbance of the cyclic activity of the thyroid acinus is not initiated by a chemical agent but by the decline in ovarian function. The resulting excess of thyrotropic hormone will, as in thiouracil goiter, produce loss of colloid and hyperplasia of the thyroid epithelium associated with hypofunction.

#### *Discussion*

(Dr. Carl V. Weller, Ann Arbor, Mich.) I would like to ask the speaker how many lymphocytes he requires in the thyroid before he designates a particular example as belonging to the group of lymphadenoid goiters.

(Dr. William Boyd, Toronto, Ont.) I regret that the speaker does not stick to the title of the paper on the program, which is the differentiation of lymphadenoid goiter from chronic thyroiditis. One of the most difficult problems we have to face is to determine the relationship between these two conditions. I hope before closing Dr. Parmley will refer to that. Does he consider these conditions entirely independent, or may one after a period of years merge into the other?

(Dr. William H. Harris, New Orleans, La.) Were follicles or germinal centers present?—I ask this, as it indicates local production. The intimate relationship of lymphoid and thyroid structures in embryonal development must also be considered. The lateral aberrant lympho-thyroid tumors are evidences of gross exaggeration.

(Dr. Hamilton Fishback, Chicago, Ill.) I would like to know if any of the patients had had previous iodine therapy.

(Dr. Wiley D. Forbus, Durham, N.C.) May I ask if in your experience there has occurred any appreciable increase in the incidence of this condition since the introduction of the sulfa drugs in therapy?

(Dr. Parmley) As regards Dr. Weller's question: the amount of lymphoid tissue necessary for this type of goiter we have found to vary to some degree, but on the average as previously stated, these glands consist of approximately one-third lymphoid tissue.

As Dr. Boyd pointed out, time did not permit us to discuss the question of differentiating lymphadenoid goiter from chronic inflammation. We intend to do this in the paper to be published. We consider the essential difference between chronic thyroiditis and lymphadenoid goiter to be the presence in the latter of large, pale eosinophilic cells, the absence of tubercle-like giant cells, and of fibrosis throughout the gland. Also, this type of goiter occurs in the female around the menopausal age. Chronic thyroiditis occurs as a rule in younger persons, with equal frequency in both sexes. We consider these conditions entirely independent and one after a period of years will not merge into the other.

We did find germinal centers quite frequently throughout our specimens. They varied in number and size and were present in all but two or three glands.

In answer to Dr. Forbus' question, I cannot speak too definitely about the frequency of this type of goiter since the advent of the sulfa drugs. In the last few years we have noticed a definite increase in this type of goiter, namely, among 500 goiters there were 14 lymphadenoid goiters, *i.e.*, 2.8 per cent.

In answer to Dr. Fishback's question, all of these patients with lymphadenoid goiter had iodine preoperatively. One patient was on Lugol's solution 6 months before operation.

#### THE PATHOGENESIS OF TUBEROUS BONE FORMATION IN THE LUNGS. Kornel L. Terplan, Buffalo, N. Y.

*Abstract.* In the course of systematic search for calcified tubercles small bony particles frequently can be encountered in the lungs, either in very small numbers (from one to four) or in larger numbers symmetrically distributed. As single structures these are known in the literature as "osteomas," the disseminated type as the tuberous form of bone formation. Such bony structures were seen in larger numbers in cases of cardiac insufficiency by Freeman-Dahl and in patients with mitral stenosis by Gross and Janker, who studied their roentgenologic appearance and increase in size over a period of from 4 to 8 years. Wells reported one such case of disseminated bone formation in mitral stenosis. The pathogenesis of this bone formation was admittedly not clear. The fibrosis of the lungs was found by Wells to be different from that in ordinary chronic passive congestion. The connective tissue increase was more focal, hemosiderin pigment deposition was slight. He thought that a combination of chronic interstitial pneumonia with chronic pul-

monary congestion might be instrumental in bringing about this tuberous ossification.

A case of mitral stenosis in a young adult with a very large number of these small, tuberous, bony structures offered a good opportunity to study their pathogenesis. In the parts of the lungs in which there was no roentgenologic evidence of bone formation, histologic pictures were seen which seemed to represent the early phases of, and the actual matrix for, this bone formation. Tuberous bone formation is, on the basis of our morphologic analysis, the result of thrombosis in the septal capillaries. Capillary thrombi were found either *in situ* within the septa, or, more often, protruding in various forms into the alveolar space, with one wall still adherent to the septum, or extending in polypous fashion into the lumen, or, within the alveolar spaces. It is easy to demonstrate morphologically the capillary thrombotic nature of these structures. Blood corpuscles and blood fluid remain finally, apparently free or suspended by a few thin threads of mesenchymal cells within the capillary membrane; in various states of hyalinization of the thrombus, mesenchymal (septal) cells can be seen attached to the surface of the detached thrombosed capillary. These mesenchymal cells are apparently concerned with the gradual formation of bone. No stone formation was seen in these capillary thrombi, in contradistinction to the usual "phlebolith" as found in the pulmonary arteries. It is suggested that such capillary thrombi in certain phases of their formation, especially when in but loose connection with the septa, might easily be forced into other parts of the lung by increased expiratory pressure, especially by coughing. They might be found in the sputum of patients in whom this tuberous bone formation can be demonstrated radiologically.

CRYSTALLINE ESTER CHOLESTÉROL AN IRRITANT. Timothy Leary, Boston, Mass.

*Abstract.* In the embedding of tissue in paraffin or celloidin, fatty substances, including cholesterol, are extracted from the tissues. Fresh tissue or *recently fixed* formalin tissue is essential for the study of crystalline cholesterol, which is visible as such only under polarized light.

Gye and Purdy (*Brit. J. Exper. Path.*, 1922, 3, 86-94; 1924, 5, 238-250) injected silica sol (colloidal silica) intravenously in rabbits and produced, under low dosage over a considerable period, cirrhosis of the liver, enlargement of the spleen, and chronic nephritis, which lesions they looked upon as characteristic of chronic silica poisoning. Rabbits fed cholesterol over long periods will develop, in addition to atherosclerosis, cirrhosis of the liver, enlargement of the spleen, and chronic nephritis. These lesions arise following the precipitation in the liver of cholesterol ester crystals. The crystals are treated as foreign bodies. They are taken over from the liver cells by Kupffer cells, are carried into the lymph and blood streams by these macrophages, and stimulate the growth of connective tissue wherever they are stored within the lipophages. The most efficient form of silica as an irritant is in crystals 1 to 3  $\mu$  in diameter (Gardner, L. U., and Cummings, D. E. *Am. J. Path.*, 1933, 9, 751-763). Cholesterol ester crystals are taken into lipophages in particles (droplets) 1 to 3  $\mu$  in diameter. Both silica and ester crystals are treated as foreign bodies, provoke phagocytosis, and stimulate the growth of fibrous tissue.

CRYSTALLINE ESTER CHOLESTEROL AND ATHEROSCLEROSIS. Timothy Leary, Boston, Mass.

*Abstract.* Evidence is presented that crystalline ester cholesterol is present in all *active* lesions of atherosclerosis. Solid crystals of cholesterol may arise in late lesions through the necrosis of lipophages carrying ester crystals and the splitting of the esters. Only advanced calcified or repaired lesions are free from crystalline cholesterol. A progressive sequence of changes in the evolution of (a) aortic and

(b) coronary sclerosis is shown. Atherosclerosis cannot be produced experimentally by wear and tear methods. Crystalline cholesterol appears to be the essential substance in human and experimental sclerosis.

### *Discussion*

(Dr. E. T. Bell, Minneapolis, Minn.) I would like to ask Dr. Leary if it is not true in human atherosclerosis that you can find occasionally small early lesions that are pale whitish, and in which, on microscopic examination, you get the impression that the edema and loosening of the tissues precede the deposit of the cholesterol. Most of us have had the idea that the cholesterol deposit was secondary to some injury of the aorta, and Dr. Leary apparently thinks that the cholesterol is deposited first and that it is responsible for the atheroma.

(Dr. Leary) The idea that cholesterol deposit in atherosclerosis is secondary to some injury of the aorta is based on the Virchow-Aschoff "imbibition theory" which has delayed recognition of the real cause of the disease. Klotz first, and Anitschkow later, insisted that the aortic intima showed no evidence of injury in the earliest lesions of the disease, which were marked by the appearance of cholesterol within macrophages in the subendothelial layer of the intima. They did not know the sources of the macrophages, which are Kupffer cells from the liver as I have shown. Earliest lesions appear as atheromatous processes in the aorta of youth and also in the ascending aorta even in age. With adequate human material it is possible to collect series of ascending aortae with crops of pinhead lesions that are colored orange-yellow in the earliest stages, paler yellow in mid-course, and gray-yellow to gray-white in later stages. These stages represent the waxing and waning of atheromatous lesions in which the cholesterol esters, after being deposited, are removed by a mechanism corresponding to that which removes the excess cholesterol from atheromatous lesions in youth. It is this mechanism which protects the ascending aorta from advanced lesions. When this mechanism is destroyed, as by syphilitic aortitis, continuous atherosclerotic lesions appear in the ascending aorta. The earliest lesions are made up of macrophages filled with cholesterol ester crystals beneath the aortic endothelium. Pale whitish lesions with edema are advanced and not the earliest lesions.

(Dr. A. R. Crane, Norfolk, Va.) I would like to ask if Dr. Leary has noticed the occurrence of hemorrhages in these atheromatous lesions with any degree of frequency, and has he had an opportunity to note any changes in the prothrombin time of these animals.

(Dr. Leary) First, in reference to hemorrhages, they are always a late phenomenon in atherosclerosis; in the coronaries we get vascularization from the coronary lumen in late lesions, and one of the means of producing thrombosis without question is vascular spasm which ruptures the capillary network and leads to hemorrhage and to fibrinoid necrosis, extending to the endothelium and causing thrombosis. Hemorrhages are a very late phenomenon under any circumstances. With reference to the prothrombin time, I have done no work on that.

(Dr. William H. Harris, New Orleans, La.) I would like to ask what Dr. Leary considers the pathogenesis in those lesions with manifest mechanical or physical relationships, such as those at the orifices of the intercostals, or as shown in industrial employment as "one leg" hyperactivity and the like. How does he link such with crystalline ester cholesterol irritation?

(Dr. Leary) The coronary arteries, notably the epicardial branches, are the most common and most important sites of atherosclerotic lesions. During systole the epicardial branches of the coronaries are distended with blood under systolic pressure, while the muscular branches are compressed by the contracting ventricles. There is a definite pallor of the contracting ventricles, notably the left. This results in a slowing of the blood in the epicardial branches, sometimes almost to stasis, so

that there is opportunity for the sticky macrophages to adhere to the wall. Similarly, at the end of the systole temporary stasis favors invasion of the intima in the ascending aorta. About the orifices of the intercostal and other branches of the aorta there is a break in the continuity of the forward movement of the blood. The swirling currents about the orifices presumably produce conditions favorable to the adhesion of the cells to the wall. In the experimental rabbit the origin of the lipophagic cells in the liver sinusoids, their phagocytosis of crystalline esters, their escape into the blood stream, their passage through the lungs, and their entrance into the aortic intima can be followed in detail. The irritant quality of cholesterol ester crystals is responsible for the progression of the disease.

TUMORS OF THE TESTIS. Nathan B. Friedman, Capt., M.C., Washington, D. C.

*Abstract.* A simplified classification of tumors of the testis has been set up, based on study of more than 800 such neoplasms collected at the Army Institute of Pathology between 1941 and 1946. Ninety-nine per cent of them fall into one of the following categories: seminoma, embryonal carcinoma, chorioepithelioma, teratoma, teratocarcinoma, or interstitial cell tumor. Serial sections of small tumors have revealed that not all monocellular neoplasms of the testis originate from teratomas. Seminoma and the embryonal carcinoma differ not only in fundamental cell type but in biologic behavior and prognosis. Consequently, the misleading term "embryonal carcinoma with lymphoid stroma," which does not designate a pathologic entity, should be abandoned. The chorioepithelioma should be considered a subvariety of embryonal carcinoma because of the tendency toward trophoblastic differentiation evident in many carcinomas and the frequent occurrence of mixed, intermediate, and transitional forms.

A new term, "teratocarcinoma," is proposed for the large group of pleomorphic tumors in which both differentiated teratoid structures and malignant elements are present. The view that the carcinomatous component of such mixed neoplasms does not necessarily arise from the teratomatous portion is supported by the fact that it is usually an embryonal carcinoma and is almost never of a specialized epithelial type. Moreover, teratoid differentiation in embryonal carcinomas does take place. Virtually all metastases of embryonal carcinomas develop as monocellular embryonal carcinomas or chorioepitheliomas. Roughly half of the teratocarcinomas which metastasize give rise to tumors with teratocarcinomatous structures and half to pure embryonal carcinomas. Choriomatous characteristics may be evident in the metastases of an embryonal carcinoma or teratocarcinoma even when they are not manifest in the primary. The immediate prognosis for embryonal carcinoma and chorioepithelioma is very bad. Since it is almost as serious for teratoma, this neoplasm should be termed "adult" and not "benign." Mixed teratocarcinoma metastasizes less readily and has a lower early mortality than either carcinoma or teratoma. The immediate prognosis for seminoma, by comparison with the other tumors, is very good.

The element from which testicular tumors other than seminoma arise possess the developmental potencies of normal germ cells, for it can differentiate into both somatic and trophoblastic tissues. It has been suggested that the seminoma represents an attempt to reproduce testicular tissue itself, a theory which is attractive but not established.

#### *Discussion*

(Dr. Nicholas M. Alter, Jersey City, N. J.) I would like to ask one question in regard to nomenclature which is rather academic. Since the ovarian dysgerminoma is identical with the dysgerminoma of the testis, why not call it dysgerminoma of the testis? It does not deserve the name "seminoma" because it does not derive from seminiferous tubules. Perhaps the time will come when these tumors can be classified into immature and mature teratomata.



(Dr. Friedman) The only reason I retained the word "seminoma" is because so many people use it, and I agree with Dr. Alter that this tumor is an identical tumor with the ovarian dysgerminoma. The term, "embryonal carcinoma with lymphoid stroma," has not been used; this is usually a seminoma, but some of the embryonal carcinomas have a lymphoid stroma. In mortality statistics the difference is amazing; only 1 per cent of patients with seminomas have died thus far, whereas 25 per cent of those with embryonal carcinomas have succumbed. I think if I can ever find out what the seminoma is, I will have a name for it; the name will suggest itself. I do not want to change it because I have nothing better to call it. The term, dysgerminoma, is equally acceptable, but I do not think they should all be called teratomas, and I am not sure where the seminoma cells and the seminoma tumor fit into the scheme.

(Dr. Alfred Plaut, New York, N. Y.) How often has a giant cell reaction in the necrotic portion of the lymphoid stroma been found, as is so frequently found in the corresponding ovarian tumor; and how often have isolated areas of cartilage been found in a tumor which looks like a seminoma?

(Dr. Friedman) I cannot give you the percentage incidence of granulomas in seminomas, but I would guess it is somewhere in the neighborhood of 15 or 20 per cent. As a matter of fact, we have a few tumors in which there was no seminoma tissue left except for an occasional isolated seminoma cell, and the whole mass was granulomatous. That is another reason for abandoning the lymphoid stroma idea, because if we would follow that we would have to say "with eosinophilic stroma," or "with epithelioid stroma," or "with granulomatous stroma," etc., as the case might be. I do not think the granulomas are invariably associated with foci of necrosis.

With regard to the finding of isolated lumps of cartilage, we have made serial sections when all of the tumor was submitted and it was small enough to section serially. Those which were too large were submitted to multiple block study. In a number of seminomas teratoid foci have appeared. In that simplified pie-shaped diagram which I showed, the teratomas associated with seminomas were combined under teratoma. That is an over-simplification for purposes of illustration, and seminoma does occur in combination with the other tumors. Seminomas in which there is a small focus of cartilage and usually some glands, may be side by side with teratomas as distinct entities. The significance of this is not clear, but such tumors would fit into the group "teratoma with seminoma."

(Dr. Joseph Tannenber, Batavia, N. Y.) I would like to ask if Dr. Friedman has any information as to the life-expectancy of the patients after surgical removal and eventual x-ray treatment of the several types of tumors of the testis mentioned in his report.

(Dr. Friedman) We are a little restricted at the present time because the follow-up through the Veterans' Administration has not been worked out yet, so that our follow-up is only the immediate follow-up. There will be a long-range follow-up through the Registries. I have only the immediate follow-up now, 6 to 12 months at Army Hospitals. After the patient has his tumor removed and gets well, either he goes home or to the Veterans' Administration. All I can tell is what happened in Army hospitals, and it may be that the next year or 5 years will change the picture. The immediate expectancy for seminoma is very good. Only 1 per cent of our 300 patients with seminomas are dead. Twenty-five per cent of the patients with embryonal carcinomas and chorioepitheliomas are dead. The surprising thing is that of those with adult teratomas almost 20 per cent are dead. The deaths are mostly from the wicked metastasizers differentiating in the direction of chorioepithelioma. What the others are going to do I cannot say, but there is a sharp dichotomy between the teratoid group and the seminoma group. When Dr. Moore came on as civilian consultant at the Institute one of the first things he

asked was "can the seminoma be a benign tumor?" It is not a benign tumor, but the disparity is so striking that it points out these differences.

**ETIOLOGIC FACTORS IN PATIENTS WITH CARCINOMA OF THE PENIS AND IN CONTROL GROUPS.** Robert Schrek, Maj., M.C., and (by invitation) Herman Lenowitz, Hines, Ill.

*Abstract.* The clinical group under investigation consisted of 139 men with carcinoma of the penis. The data on the control groups were obtained from the records of patients with cancer and from interviews with men in the hospital. The percentage of colored patients was 28.1 per cent for the men with penile cancer and 6.4 to 7.4 per cent for the control groups. None of the men with cancer of the penis were circumcised as babies, but 12.8 per cent of the white and 17.9 per cent of the colored control patients were circumcised early in life. Circumcisions during boyhood and manhood were as frequent in the clinical as in the control groups. Syphilis occurred four times and gonorrhea two times as frequently in men with penile lesions as in the control groups. The incidence of both venereal diseases was twice as high in colored as in white patients. Circumcised and noncircumcised men had the same incidence of venereal disease. It is estimated that colored and white men free of venereal disease had equally low incidence rates of carcinoma of the penis. Colored men with syphilis or gonorrhea had a much higher incidence of penile cancer than white men with venereal disease. The high frequency of carcinoma of the penis in colored men could not be attributed to differences in incidence of circumcision or venereal disease or to racial immunity, but may possibly be due to differences in sex hygiene.

#### *Discussion*

(Dr. Alfred Plaut, New York, N. Y.) There is a curious similarity in the occurrence of carcinoma of the penis and carcinoma of the uterine cervix. As most of you know, carcinoma of the uterine cervix is rarer in Jewish women than in other populations. This applies to numerous countries; the statistics are available for the United States, for Austria, Germany, and England. It would be extremely interesting if statistical studies such as that by Dr. Schrek could be carried out in a parallel way for carcinoma of the uterine cervix.

(Dr. Joseph Tannenbergh, Batavia, N. Y.) I should like to underline the warning of Dr. Plaut; there are so many other factors which are more important in the pathogenesis of penile carcinoma than this obvious mechanical factor of circumcision. In addition to the type of tumor mentioned by Dr. Plaut, I would like to mention carcinomas of the gallbladder, bile duct system, and pancreas, the incidence of which is quite different in different races or strains of the white population. Here in this country, particularly in the State of New York, I have found during the last decade that carcinoma of the gallbladder and the bile duct system is a rather rare disease, much less frequent than carcinoma of the pancreas. In Germany, however, where I saw about 2000 autopsies annually over a period of 12 years, carcinoma of the gallbladder and of the common bile duct is a very common disease, at least ten times as frequent as carcinoma of the pancreas, while it is almost the reverse in this country.

(Colonel J. E. Ash, Washington, D. C.) I am wondering if the essayist meant to imply that circumcision and sex hygiene in themselves would prevent carcinoma of the penis. It is certainly true that this tumor is almost unknown among those who practice circumcision and that it is very common in China where circumcision is not practiced and also where personal hygiene is unknown among the poorer classes. Smegma is a mild carcinogen. The important factor, therefore, is not necessarily circumcision or sex hygiene as much as it is the use of soap and water.

(Dr. Plaut) Colonel Ash mentioned the fact that smegma is a carcinogenic

substance. I do not know how he knows about that. I made the same assumption years ago. The dorsal skin of numerous mice was treated with human or equine smegma. The results are not as yet properly tabulated, but two carcinomas grew at the site of treatment, and several papillary lesions were observed. Whether the results are significant enough to call smegma an experimental carcinogenic agent I do not yet know.

ANGIOMATOID CHANGES IN THE GENITAL ORGANS WITH AND WITHOUT TUMOR FORMATION. Robert P. Morehead, Winston-Salem, N. C.

*Abstract.* A characteristic group of tumors of unusual structure which occur in the genital tract has been described in the literature under a variety of names. These tumors have been designated as adenomas, adenocarcinomas, mesotheliomas, mixed leiomyomas and lymphangiomas, and adenomatoid tumors. These neoplasms are characterized by vacuolization of incompletely differentiated mesenchymal cells, which ultimately results in the formation of spaces lined by cells of unusual structure. Regarding the exact nature of these cells, there is considerable difference of opinion. In this paper it is shown that these same changes take place in the genital organs apart from neoplasia, and evidence is presented to support a histogenetic relationship with vascular differentiations of the mesenchyme.

*Discussion*

(Dr. Alfred Plaut, New York, N. Y.) I fully agree with Dr. Morehead that these tumors are angiomatous, and that all the changes which make the lining cells look like epithelium are secondary. Lymphangioma in genital organs is not so rare. In my own material I have seen five in the last 10 years, and I have been shown numbers by other pathologists. There is a similarity in localization: in the epididymis in the male and in the tube in the female—certainly not a morphologic parallelism, but a physiologic one. I have no explanation for that. The lymphoid foci mentioned are not merely accumulations of lymphocytes; a reticulum stain shows a reticulum exactly as in lymphoid tissue. This, in my opinion, is another strong argument in favor of interpreting all these tumors as lymphangiomata no matter how much some of the lining cells may look like epithelium. Dr. Morehead has not mentioned the most spectacular lymph-vessel tumor in the genital tract, namely, the lymphangiocystic fibroma of the uterus. This very rare tumor—I have seen only one—can reach the size of a full-term gestation.

HISTOGENESIS OF HYDATIDIFORM MOLE. Nicholas M. Alter, Jersey City, N. J.

*Abstract.* Within the limits of this paper only the salient facts are presented. At the Margaret Hague Maternity Hospital 60 cases of true hydatidiform mole were observed in the last 15 years among 83,225 deliveries and 2,750 abortions, giving a proportion of 1 in 1,400 pregnancies.

Case 2 illustrates the clinical and pathologic findings of a benign mole. Microscopically, villi were all without blood vessels. Over the surface anaplastic proliferation of trophoblasts was seen. The glycogen content in the benign type was marked. Mostly over the surface of Langhans' cells, syncytial giant cells were seen with vacuolated cytoplasm which often took fat stains. In the stroma regressive changes were seen with cyst formation; near the surface trophoblasts invaded the stroma and might be found at a distance.

The malignant course is typified by case 41. Malignant chorionepithelioma was found in the uterus removed 7 weeks after the mole. Upon microscopic examination a diffuse proliferation of small cuboidal cells was seen; there was no structural arrangement; necrosis and hemorrhage were extensive; the cells showed anaplasia, some mitotic figures, and pyknotic nuclei; they contained no glycogen.

Hydatidiform moles removed superficially or passed give a variety of histologic pictures depending on (1) their situation in utero, (2) regressive changes due to retention in utero, and (3) changes after removal. Hydatidiform moles *in situ* naturally can give additional information. Such a uterine specimen was removed in case 7. Frozen section of the uterus was made in order not to disturb the contents. On cross section a rather orderly arrangement was observed. Microscopic examination revealed more exuberant epithelial proliferation near the blood supply at the base than in the interior. Where trophoblasts were less proliferative and more differentiated they formed lacunae obviously for fluid exchange replacing vascularization; such channels could be observed over the surface as well as within the villi.

To extend this structural study, suitable specimens were pieced out from physiologic solution. One such preparation was 30 cm. in length. Microscopic sections of the alternating blebs and stems had epithelial covering of trophoblasts, which in places invaded the deeper layers and seemed flattened at the lining of the cavity. They formed also partly hyalinized masses within the stems. The blebs of the mole were analogous to retention cysts with retained material of metabolism due to complete lack of drainage. In material from abortions the avascular villi showed marked trophoblastic proliferation with bud-like outgrowth of branches.

*Summary.* A true hydatidiform mole is an epithelial neoplasm of the chorionic trophoblasts with secondary cystic changes of the stroma in uniovular pregnancy. This epithelial proliferation shows various degrees of the histologic changes found in malignant growth: anaplasia and invasion, which are, however, intrinsic characteristics of these embryonal cells. Evidence seems to indicate that these proliferative changes are due to the disappearance of the allantoic circulation. Cystic changes of chorionic villi due to regression of the final vascular system are false moles, as observed in early abortion, ectopic and other uniovular pregnancies.

#### EXAMINATION OF SPUTUM FOR CANCER CELLS AND PARTICLES. REVIEW OF LITERATURE AND CASE REPORTS. Siegfried Tannhauser, Buffalo, N. Y.

*Abstract.* Examination of sputum for cancer cells has been largely neglected, although results in suitable cases are surprisingly unequivocal. The examination is done in smears or, as in the cases of this paper, in paraffin embeddings of the whole sputum collected over several days in acidified formaldehyde solution, which clumps and solidifies the mucoid sputum into relatively firm matter easily dehydrated and embedded in the usual way. This method allows making serial sections of the sputum. Ten cases with positive results are presented, verified either by biopsy or autopsy. It appears that shedding of cells and particles occurs predominantly in the small-celled and alveolar types of pulmonary carcinoma. Secondary malignant neoplasms are also amenable to diagnosis, if they produce ulcerative lesions in the bronchial tree.

#### CARCINOMA OF THE THYROID OCCURRING IN A CASE OF DIFFUSE TOXIC HYPERTROPHIA TREATED PREOPERATIVELY WITH THIOURACIL. A. R. Crane and (by invitation) R. L. Payne, Norfolk, Va.

*Abstract.* The marked epithelial proliferation produced in the thyroid by thiouracil and the fact that thiourea in combination with 2-acetaminofluorene produces carcinoma of the thyroid in rats raise the question as to the carcinogenic properties of thiouracil in man. Because of this the following case is reported. A white female, 56 years old, with a typical history of hyperthyroidism and showing the clinical findings of this condition, had a subtotal thyroidectomy done. Prior to operation she was treated with thiouracil over a period of 6 weeks, receiving 10.1 gm. in all. The thyroid tissue removed weighed 17.5 gm. It showed one small

(1 cm.), white, firm area in each lobe. The remainder of the gland was normally lobulated and brown. Sections of the small areas showed an irregular glandular and papillary growth with large cells, atypical nuclei, and prominent nucleoli. These have been interpreted as small areas of carcinoma. The remainder of the tissue showed a typical picture of involuting diffuse hyperplasia. The facts that thiourea in combination with 2-acetaminofluorene produces carcinoma of the thyroid in rats and that carcinoma of the thyroid is rare with diffuse hyperplasia suggest that there may have been an association between thiouracil and the development of carcinoma in this case. The validity of this hypothesis will be determined by subsequent observations.

### Discussion

(Dr. Edmund Mayer, Stamford, Conn.) It is difficult to find any similarity of mechanisms in a thyroid carcinoma in a thiouracil-treated patient and the formation of those thyroid tumors which we observe in thiouracil-treated rats. In the experimental rat, thiouracil acts on a normal thyroid, while it acts on a hyperactive gland in the human patient. The production of thyroxin is depressed from normal to sub-normal in the rat. As a consequence the anterior pituitary body produces more thyrotropic hormone, which has the following effect: The thyroid remains unable to correct its functional deficiency, but it can and does react with morphologic activation, first within the regular histologic pattern, and finally, after about 8 months of continuous administration, with the formation of adenomas and occasional carcinomas. In the human patient an excessive functional and morphologic activity of the thyroid is present before treatment with thiouracil. When, through this treatment, a more or less normal condition has been obtained, why should the anterior pituitary gland start to produce more thyrotropic hormone than before? If so, why should the thyroid be activated morphologically beyond its condition before treatment, and why should the histologic pattern be lost now, when it had been maintained during the highest degree of hyperthyroidism? In other words, the morphologic processes in the thyroid develop in opposite directions when a normal rat and a human patient are treated with thiouracil. The more we can be certain that the thyroid tumors in rats are caused by thiouracil, the less we can assume that a thyroid carcinoma has been caused by thiouracil treatment of a patient with hyperthyroidism.

(Dr. Oscar B. Hunter, Washington, D. C.) Have you made any cholesterol studies in this case?

(Dr. Crane) I believe that a cholesterol study was not done on this patient.

THE IN VIVO SENSITIVITY TO STREPTOMYCIN OF RECENTLY ISOLATED STRAINS OF HUMAN TUBERCLE BACILLI. William H. Feldman and (by invitation) H. Corwin Hinshaw, Rochester, Minn.

*Abstract.* The original observations on the ability *in vivo* of streptomycin to exert a successful deterrent effect on experimental tuberculosis were made with infections established by the laboratory strain of tubercle bacilli H 37 r v. The importance clinically of information regarding the *in vivo* efficacy of streptomycin against previously uncultured strains of tubercle bacilli obtained directly from patients with tuberculosis is obvious. Ten experiments were done using (1) seven freshly isolated strains of tubercle bacilli obtained by gastric aspiration of 7 patients with severe and progressive pulmonary tuberculosis; (2) two strains of bovine tubercle bacilli, and (3), for comparison, H 37 r v. Each strain of tubercle bacilli was used to inoculate subcutaneously 14 guinea-pigs. Starting 2 weeks later, 8 animals in each group were treated daily with streptomycin. Treatment continued for 54 days. The experiments were terminated on the 68th postinfection day. The re-

sults indicated quite definitely that streptomycin was equally effective against the infection produced by each of the ten strains of tubercle bacilli. Although practically all of the untreated controls in the respective groups showed a severe generalized and uninhibited disease, the disease in the treated animals was in most instances not demonstrable or was limited to the site of inoculation.

THE EFFECT OF STREPTOMYCIN ON THE HISTOPATHOLOGY OF HUMAN TUBERCULOSIS. Archie H. Baggenstoss, William H. Feldman, and (by invitation) H. Corwin Hinshaw, Rochester, Minn.

*Abstract.* There is convincing evidence that streptomycin exerts a profound regressive action on the morphologic aspects of well established tuberculosis in guinea-pigs. Material from 5 fatal cases of tuberculosis in human beings treated with streptomycin was studied to determine if similar morphologic signs of therapeutic effect could be recognized. For control purposes an unselected group of similar cases that had not been treated with streptomycin was also studied. The observations on the few cases examined suggest that following a period of treatment with streptomycin lasting several weeks or more, tuberculous lesions in the spleen, liver, and especially the lungs are more discrete and less cellular. Many of the lesions become atrophic, fibrotic, and hyalinized. Caseation is minimal or absent. In many instances the lesions eventually lose their characteristic tuberculous identity and appear as nonspecific granulomatous foci.

The findings in the central nervous system were unique. The meningeal lesions in some cases were regressing or were no longer demonstrable, although in some instances an apparently uninhibited, focal tuberculous encephalitis was present. The absence of meningeal lesions in cases in which tubercle bacilli had previously been demonstrated in the spinal fluid would seem to be a significant observation.

### *Discussion*

(Dr. Kornel L. Terplan, Buffalo, N. Y.) The hematogenous tubercle has, in general, the tendency to heal. In cases with miliary tuberculosis as the cause of death we see a good number of hematogenous tubercles in the liver or in the lungs with a very marked healing tendency. Only in the massive overwhelming type of acute miliary tuberculosis, especially in very early life, combined with hemorrhages in caseated lesions of the primary complex, are the hematogenous tubercles actually miliary necroses. In the majority of children with miliary tuberculosis a healing tendency of the hematogenous tubercle in various organs could be noticed. The cause of death in most of these was tuberculous meningitis. It might be of interest to evaluate the results described by Dr. Feldman and associates in connection with the duration of the disease and the type of infection (whether acutely overwhelming or the more usual type of progressive miliary tuberculosis).

(Dr. Howard C. Hopps, Oklahoma City, Okla.) I should like to ask why so many of the experimental animals that received streptomycin died from causes which were apparently not related to tuberculosis.

(Dr. Feldman) That is a good question, and I hoped that some one would ask it. We lost some, it is true. Most of these animals had at necropsy a massive abdominal hemorrhage. We were quite concerned for a while, and were at a loss to account for these premature deaths. We finally thought of the possibility that these animals may have been subjected to injudicious restraint when they were being injected with streptomycin. We drew this to the attention of our assistants. Since then few deaths from abdominal hemorrhage have occurred. This may or may not be the answer.

(Dr. William H. Harris, New Orleans, La.) I would like to ask whether this was

a "prophylactic" experiment, or was it established that such animals had already developed the disease.

(Dr. Feldman) I tried to make it clear that we injected the animals with 0.1 mg. of the organism and waited 2 weeks before treatment, and then they were treated daily with 6,000 units of streptomycin given in divided doses. On this basis it was a therapeutic and not a prophylactic experiment.

(Dr. Harris) Were any of the animals of the group to be treated sacrificed at the 2-week interval in order to ascertain the degree of lesion present at that period?

(Dr. Feldman) One or two of them had died with tuberculosis of the liver or spleen at that time; and even those which died within 10 days showed microscopically some tuberculosis of the spleen.

INFLUENCE OF PENICILLIN IN SUBACUTE BACTERIAL ENDOCARDITIS. Robert A. Moore, St. Louis, Mo.

*Abstract.* Healing in subacute bacterial endocarditis includes four basic processes: covering the vegetation by fibrous tissue; phagocytosis and destruction of the bacteria; organization and calcification of the necrotic central part of the vegetation; and endothelialization of spaces within the vegetation. Penicillin, by controlling the further growth of bacteria, allows healing to occur more rapidly. Treatment should include other agents known to influence wound healing, notably maintenance of an adequate level of plasma albumin and a sufficient intake of vitamins.

*Discussion*

(Dr. Benjamin J. Clawson, Minneapolis, Minn.) I would like to ask Dr. Moore if he has seen recurrence after treatment. The reason I ask is that I have seen several cases which have been treated and clinically were apparently cured. The blood stream became free and all symptoms disappeared; and 2 patients have died from other causes such as heart failure or mycotic aneurysms in the brain. In those heart valves I found, as he did, that the organisms were gone. The valves were in the process of healing. In some of my cases I have found a typical picture of acute rheumatic endocarditis of the valve. That led me to believe that we have a rich field there for re-infection and re-occurrence of bacterial endocarditis. I have not seen recurrence in the few cases I have observed.

(Dr. Moore) I cannot answer Dr. Clawson's question, inasmuch as my experience with this disease is largely limited to the study of the pathologic anatomy of the lesions of the heart. However, in the Barnes Hospital I can say that there have been a number of patients with recurrences similar to those cited by Dr. Clawson. I have not observed in these 24 cases any evidence of active rheumatic fever after the process of healing had occurred.

(Dr. Howard T. Karsner, Cleveland, O.) Experience has shown that in clinical practice large doses of penicillin are necessary for satisfactory treatment of subacute bacterial endocarditis. Did all the patients reported by Dr. Moore receive doses of penicillin which, at the present time, would be regarded as therapeutically adequate?

(Dr. Moore) No, Dr. Karsner, they did not. Some of the patients treated in 1942 received relatively small amounts of penicillin. From a study of these slides it is difficult to make any definite recommendation as to the dosage, but I may say this: A dose of penicillin should be given which will maintain a blood level to which the organism in that patient is sensitive over a sufficient period of time to bring about healing of the vegetation. That is the general principle. To be more specific, I think the dosage is never less than 200,000 to 400,000 units in 24 hours, given intravenously or intramuscularly at least every 2 hours, and for a period of time somewhere between 1 and 4 months.

THE EFFECTS OF RADIOACTIVE PHOSPHORUS ( $P_{32}$ ) ON THE MALIGNANT LYMPHOMAS. William R. Platt (by invitation), St. Louis, Mo.

*Abstract.* A study of the cellular changes occurring in the tissues of 35 patients treated with radioactive phosphorus is reported. The various types of leukemia constitute the majority of the cases studied. However, several lymphosarcomas and leukosarcomas, one case of Hodgkin's disease, one Ewing's tumor, and one melanoma were also included in the series. Quantitative measurements of residual  $P_{32}$  in the autopsied organs are correlated with the histopathologic changes observed.

*Discussion*

(Dr. Howard T. Karsner, Cleveland, O.) The possibility of injury by the use of  $P_{32}$  as indicated by Dr. Platt's report, together with reports of lack of benefit in leukemic disorders by treatment with  $P_{32}$ , raise the question as to whether this agent may do more harm than good.

THE EFFECT OF BAL THERAPY ON THE PATHOLOGY OF SYSTEMIC CADMIUM POISONING. Arthur M. Ginzler, Maj., M.C., and (by invitation) A. Gilman, Maj., Sn.C., F. S. Philips, 1st Lt., Sn.C., R. P. Allen, and E. S. Koelle, Edgewood Arsenal, Md.

*Abstract.* The development of BAL (2, 3-dimercaptopropanol) has led to great advances in the understanding of the toxicologic and pharmacologic mechanisms involved in heavy metal poisoning. Evidence has accumulated that the therapeutic action of BAL depends on its ability to compete for the intoxicating metal against vital SH-containing protoplasmic constituents, apparently enzymatic proteins, with the formation of relatively nontoxic mercaptides.

Although cadmium poisoning is not of common clinical occurrence, it notably exemplifies the mechanisms involved in this group of agents as a whole. The intravenous administration to rabbits of lethal amounts of cadmium chloride results in acute toxic death, in most instances within 24 hours, accompanied by moderate central and midzonal hepatic necrosis, lymphorrhexis, visceral congestion, and, with rare exception, *insignificant or no renal lesions*. In striking contrast, when the injection of the cadmium salt is accompanied by the prophylactic administration of BAL (1 minute prior to the cadmium), there is an exaggeration of the hepatic and lymphoid damage, and more important, the development of severe renal damage manifested by extensive tubular necrosis, chiefly of the proximal convoluted tubules. Gilman *et al.* (in press) have explained these effects on the basis of an *in vivo* 1:2 combination of cationic cadmium and BAL to form a soluble  $Cd(BAL)_2$  complex, excretion of which is more greatly (or more rapidly) effected by the kidney than is that of cadmium in untreated animals; they postulate that *in vivo* mechanisms result in dissociation of this complex within the renal tubular epithelium with the consequent accumulation within the kidneys of far greater amounts of cadmium than is the case in the untreated rabbits. They have submitted evidence for the actual *in vitro* formation of such a complex, as well as another, insoluble, 1:1 Cd-BAL complex. Further information has been gathered from the pathologic effects of injection of the actual preformed complexes; the pathology of the insoluble Cd-BAL resembles that of untreated cadmium, whereas the pathology of this complex plus the prophylactic administration of BAL, as well as the pathology of the soluble  $Cd(BAL)_2$  with no further treatment, resembles that of cadmium plus prophylactic treatment with BAL.

Actually, therapeutic administration of BAL (at an interval after the injection of the cadmium salt), as compared with the prophylactic administration of BAL, results in definite amelioration of both the hepatic and renal lesions. A proffered



explanation (Gilman *et al.*) is that the delay in therapy may permit the rapid irreversible tissue fixation of nontoxic amounts of cadmium. Such a mechanism would result in the mobilization of lesser amounts of cadmium to the kidneys and liver, as compared with prophylactic treatment, and consequent lesser degree of injury to the kidneys and liver.

ON THE NATURE AND GENERAL PATHOLOGIC SIGNIFICANCE OF GRANULOMATOUS INFLAMMATION. Wiley D. Forbus,\* Durham, N. C.

*Abstract.* In this paper the author deals with a general pathologic consideration of the subject of granulomatous inflammation, with particular emphasis upon the specific chemotactic properties of certain etiologic factors for the cells of the reticulo-endothelial system. A reorientation of the pathogenic problems involved in the granulomatous diseases, particularly those produced by some of the more important fungi, is attempted. A brief statement of the general pathologic significance of granulomatous inflammation is given in the concluding section of the paper, which will be published in full in the American Lecture Series, published by Charles C. Thomas.

TISSUE CHANGES IN FUNGUS DISEASES. Roger D. Baker, Birmingham, Ala.

*Abstract.* When the microscopic appearances of fungus diseases were tabulated with respect to the degree of suppuration, macrophage and giant cell response, caseous necrosis and fibrosis, the following observations were made:

1. Several of the deep fungus infections, such as blastomycosis (North and South American), coccidioidomycosis, sporotrichosis, and moniliasis (when a deep infection), show all of these tissue changes.
2. Others of the deep infections, such as actinomycosis, nocardiosis, and maduromycosis, show all of these changes except caseous necrosis.
3. A few of the deep mycoses are not usually attended with suppuration.
4. Mycoses may run their entire course with only acute necrosis or acute inflammation.
5. The superficial fungus infections often have no inflammatory response, but may have acute or chronic inflammatory response.

It is concluded that chronic suppuration with fibrosis is probably the most general tissue change in deep fungus infections and that the neutrophil is more usually the primary reacting cell. In some instances, however, the macrophage and giant cell may be the primary reacting cells. Tissue changes in fungus infections represent response to proliferating and dying foreign bodies, probably in some instances together with hypersensitivity to the presence of the endotoxin.

BRUCELLOTIC OSTEOMYELITIS OF ILIUM AND SCAPULA WITH GRANULOMAS OF LIVER AND GALLBLADDER. Leo Lowbeer (by invitation), Tulsa, Okla.

*Abstract.* A 63-year-old dentist contracted brucellosis by drinking raw milk from aborting cows, apparently infected through contact with lame brucellotic hogs. Brucellosis presented a chronic, recurrently febrile course for  $2\frac{1}{4}$  years, after which time soft tissue "abscesses" gradually developed, one in the left gluteal, one in the sacral, one in the right inguinal, and one in the left axillary region. Roentgenograms showed destructive lesions in both alae ilii and in the left scapula. Incision of all four abscesses yielded cheesy purulent material from which *Brucella suis* could be grown in pure culture. Inoculation of a guinea-pig resulted in typical brucellotic abscess-like granulomas in the internal organs within 8 weeks, from which

\* By invitation of the Council.

*Brucella suis* could be cultured. The strain was found penicillin-susceptible in a concentration of 1.4 units per cc. Microscopic examination of the necrotic material showed granulation tissue with acidophilic histiocytes, occasional giant cells, lymphocytes, extensive necrosis, destruction of the cortex of the os ili, and osteosclerosis alternating with osteoporosis. The abscesses drained for many months despite penicillin treatment. One year later the patient was operated upon for symptoms of cholecystitis; a grossly normal appearing gallbladder was removed and a specimen was taken for biopsy from a grossly normal liver. The liver showed many round granulomas, which were also found in the wall of the gallbladder. The bile was sterile. Several months later the sinuses were still draining and yielding *Brucella suis* which caused fatal purulent *Brucella* peritonitis and septicemia in inoculated guinea-pigs within 1 week. Roentgenograms of the chest showed multiple shadows in the right lung and effusion in the left pleura, found to be clear exudate. Bone lesions are unchanged in the roentgenograms. The strain was found to be inhibited by streptomycin in concentration of 1 unit per cc.

The case is presented (a) to demonstrate again the granulomatous nature of brucellosis which, although known in the guinea-pig since the investigations of Theobald Smith and Fabyan in 1912, has been shown to exist in man only during the last decade on the basis of a few necropsy reports; (b) to demonstrate the virulence of the porcine strain with its tendency to necrosis and "abscess" formation; (c) to show for the first time the microscopic appearance of brucellic osteomyelitis in man, which previously had been diagnosed only on the basis of clinical, roentgenologic, and bacteriologic findings.

*Note.* Cultures were checked by Dr. I. H. Borts, Iowa State Laboratory; penicillin assay by Dr. C. S. Keefer, National Research Council; slides from bone, liver, and gallbladder by Dr. W. D. Forbus.

#### GRANULOMATA OF UNKNOWN ETIOLOGY ASSOCIATED WITH PERIARTERITIS NODOSA. REPORT OF TWO CASES. Tobias Weinberg, Baltimore, Md.

*Abstract.* The first case is that of a male, aged 38, with a history of sinus trouble for 10 years. He developed bronchiectasis for which a lobectomy was performed 4 years before death. He was admitted to the hospital because of bleeding from the nose, and was found to have numerous ulcers of the nose and mouth. He died suddenly 3 weeks after admission. The autopsy revealed ulcerations in the mouth, trachea, and bronchi; large scattered areas of consolidation in both lungs; splenomegaly with areas of infarction, and swollen kidneys containing numerous petechial hemorrhages and scattered areas of infarction. Microscopically, the areas of consolidation in the lungs showed a chronic granulomatous reaction containing areas of necrosis and numerous giant cells. In most instances they surrounded blood vessels. These and other blood vessels showed a typical picture of periarteritis nodosa. Similar granulomata were found in the trachea, bronchi, spleen, tongue, and kidneys. Periarteritic lesions were also present in the spleen and kidneys.

The second case was a female, aged 50, who gave a history of pain in the legs and arms of 3 weeks' duration. There was a history of pleurisy 6 months before. Physical examination revealed a maculopapular rash over the arms, face, and trunk. The patient died 3 days after admission. Autopsy revealed lesions in the lungs similar to those in case 1. The kidneys were swollen and exhibited numerous petechial hemorrhages. A ruptured aneurysm of a branch of the renal artery was present near the lower calyx of the left kidney. Microscopically, the picture was the same as in case 1, but showed periarteritic lesions in the heart as well as in the lungs, spleen, kidneys, and gallbladder. Granulomatous lesions were present in the lungs, liver, spleen, and kidneys. /

No bacteria were stainable in the granulomata in either case, nor have fungi or parasites been demonstrated. The remaining possibility is that a virus is the causa-

tive agent. Periarteritis nodosa is apparently a terminal development, lending support to the thesis of hypersensitivity as the cause of periarteritis nodosa.

### Discussion

(Dr. G. Lyman Duff, Montreal, Que.) Dr. More, Dr. McMillan, and I have recently studied a series of 375 autopsied cases in which it was known that considerable doses of the sulfonamides had been given during life, with a view to searching for lesions that might be attributed to the sulfonamide therapy. In 22 of the 375 cases, lesions were found of a kind that I shall mention in a moment which were attributable, we felt, to the sulfonamide therapy on the grounds of exclusion of other possible etiologic agents and on the basis of identity between the lesions in the 22 cases studied and the lesions described in experimental animals treated with sulfonamides. The most frequent lesion was a granulomatous one which was encountered in 13 of the 22 cases. It was found most often in the heart, liver, or kidneys, less frequently in other organs. In one case only microscopic granulomas were found in the lung and in the wall of a bronchus. Such granulomatous lesions have been described before as being associated with sulfonamide therapy. They have also been produced in experimental animals by the administration of sulfonamides. In 7 of our cases an acute necrotizing arteritis was found. This, too, has been ascribed by Rich to sulfonamide sensitivity. Amongst the 22 cases, 4 had both lesions in common: the granulomatous lesions and the necrotizing arteritis, closely comparable with periarteritis nodosa. Whatever may be regarded as the etiology or pathogenesis of these lesions, it seems to me that this evidence gives strong support to the idea that the granulomatous reaction encountered in some cases of periarteritis nodosa belongs to the same disease or, rather, is part of that disease. Our observations also support the view that all of these lesions are of allergic nature. In any event, we have interpreted them in that sense.

(Dr. William Boyd, Toronto, Ont.) I am glad that Dr. Duff has referred to the association of the granulomatous lesions with arteritis, or, rather, with arterial lesions of various kinds, especially allergic in type. I also have seen examples of granulomatosis in sulfonamide poisoning. Recently I have encountered a case of disseminated lupus erythematosus with very characteristic collagenous degeneration in the glomerular tufts and also in the vessels of the lung, and in association with these changes in the lung there were beautiful granulomata extremely like sarcoid lesions. These were situated in relation to arteries. This general discussion of Dr. Weinberg's paper would suggest the addition of allergic granulomata to Dr. Forbus' classification, although they might be included in the third group of unknown etiology.

(Dr. Weinberg) I would like to point out that cases showing the same granulomatous reaction with particular involvement of the lungs and upper respiratory tract and unassociated with periarteritis nodosa have been reported in the literature as far back as 1906. Furthermore, the cases reported in 1939 by Wegener, in which the granulomatous reaction was associated with periarteritis nodosa, did not receive any sulfa drug therapy.

### PERINEURITIC AND POLYMYOSITIC GRANULOMATOUS NODULES IN RHEUMATOID ARTHRITIS. Gabriel Steiner, Detroit, Mich.

*Abstract.* Seen from a pathologic viewpoint, rheumatoid arthritis is a misnomer. In this disease arthritic changes may be very spectacular *clinically*, but *nosologically* they do not represent the most significant aspect of the disease. The pains and the muscular atrophy in cases of rheumatoid arthritis, as well as the peculiar bilaterally symmetrical distribution, point to an involvement of the peripheral nervous system

and of the skeletal muscles. So a few years ago, upon the suggestion of Dr. Hugo Freund, an anatomopathologic investigation of the nervous system of cases of rheumatoid arthritis was inaugurated. By courtesy of Dr. S. E. Gould, Wayne County General Hospital, and of Dr. Plinn F. Morse, Harper Hospital, autopsy material (peripheral nerves and muscles) and skeletal muscles taken for biopsies were obtained. Altogether 20 cases of typical rheumatoid arthritis were examined.

The peripheral nerves and muscles examined were brachial plexus, ilio-inguinal, femoral, and tibial nerves; deltoid, triceps, gastrocnemius, and rectus abdominis muscles. The nodules were sharply circumscribed. They were located exclusively in the perineurium of peripheral nerves and in the perimysium of the skeletal muscles. The cells composing the nodules were lymphocytes and plasma cells, the latter located more in the periphery. There were some epithelioid cells or macrophages, but no giant cells or Anitschkow's myocytes. In some cases a definite muscular atrophy as a consequence of the inflammatory nodules was seen. There was a marked increase of collagenous connective tissue, but proliferation of reticulin fibers was not seen. Occasionally a perineuritic nodule was seen around an intramuscular nerve bundle. Inflammatory infiltration of vascular walls was rare and seen only in the perimysium.

In summary, three points should be emphasized:

1. The nodular inflammation is not of a pure granulomatous type in so far as epithelioid cells or macrophages are inconspicuous and there is no necrosis in the center. Collagenous connective tissue proliferation is seen between the inflammatory cells (lymphocytes and plasma cells).
2. The specificity of the findings in the peripheral nerves and muscles has been established by a great number of control examinations (autopsy material, amputations, muscles taken for biopsy).
3. The nodules in the perineurium of the peripheral nerves and in the perimysium of the skeletal muscles represent a new link in the chain of lesions which indicate the *systemic* nature of the disease.

#### Discussion

(Dr. Benjamin J. Clawson, Minneapolis, Minn.) Stimulated by the work of Dr. Steiner and his colleagues, I was able to get specimens for biopsy from the deltoid muscles of about 70 cases of chronic rheumatoid arthritis, and in more than half of these cases I found lesions similar to those which he described. I also was able to get muscles from a few cases of acute rheumatic fever and found the lesions in them, and likewise in one case with a healed rheumatic valvular deformity I found the lesion. I thoroughly agree with Dr. Steiner that the term rheumatoid arthritis should include and mean more than the arthritis; it should include myositis or fibrositis.

(Dr. B. Black-Schaffer, Durham, N.C.) We have had an opportunity to study a case of dermatomyositis and scleroderma recently, and I would like to point out that very similar lesions were found in both instances. In a case of myasthenia gravis associated with thymoma we also found collections of lymphocytes in the skeletal muscles.

(Dr. Steiner) Dr. Black-Schaffer spoke of having examined one case of dermatomyositis. In our control material of over 300 cases we had only one case of dermatomyositis, certainly not enough to establish a clear-cut conclusion for differentiation. However, in our one case the distribution of the inflammatory process was diffuse and not nodular. This process was spread from the extramuscular tissues into the muscles. The perineurium was not involved. If this difference could be found in every case, it would be easy to differentiate the pathologic picture of dermatomyositis from that of nodular polymyositis in rheumatoid arthritis.

We had two cases of myasthenia gravis in our control material: In one we did

not find any lymphocytic infiltration, and in the other we found some—and this is the difference—located between the individual muscle fibers, and even between individual muscular fibrillae. Another difference is the peculiar nodular type in rheumatoid arthritis contrary to the less demarcated type in the lymphorrhages of myasthenia gravis. A third difference is that the lymphorrhages are composed only of lymphocytes; there are no plasma cells, no macrophages, and no connective tissue proliferation such as are seen in the muscle nodules of rheumatoid arthritis.

THE VENEREAL GRANULOMAS OF THE PENIS. N. B. Friedman, Capt., M.C., and J. E. Ash, Col., M.C., Washington, D. C.

*Abstract.* Although the histologic appearance of the primary penile venereal granulomas is fairly characteristic, it is not generally appreciated that microscopic examination of these lesions may contribute information of diagnostic value.

Granuloma inguinale bears a superficial resemblance to syphilitic chancre, but the presence of many small clusters of polymorphonuclear leukocytes amidst a dense infiltrate of round cells is strongly suggestive of granuloma venereum. Donovan bodies, which are recognizable in routine hematoxylin and eosin preparations, are rendered conspicuous if the section is impregnated with silver. The bodies must be differentiated from the granules of mast cells, especially when a Giemsa stain is employed.

The tuberculoid granulomas of lymphopathia venereum are not as unmistakable when they occur on the penis as when they are encountered in the inguinal lymph nodes, but aggregations of epithelioid and giant cells in an inflammatory lesion of the penis, especially when arranged about irregularly shaped necrotic or suppurative foci, should be strongly suggestive of lymphogranuloma inguinale.

Chancroid has a less specific histologic appearance than the other penile granulomas. The early lesion is an edematous plaque, superficially ulcerated and covered by a thin layer of leukocytes, debris, and organisms. The zone beneath shows a disproportionately scanty infiltration of inflammatory elements which tend to aggregate about blood vessels. In older lesions angiitis and periangiitis dominate the picture; the lamellated perivascular and intramural rings of round cells and the absence of accompanying diffuse infiltration are characteristic of late chancroid.

THE HISTOLOGIC DIAGNOSIS OF CHANCROID AND LYMPHOGRANULOMA VENEREUM AS SEEN IN SPECIMENS FOR BIOPSY FROM GENITAL LESIONS.\* Walter H. Sheldon and (by invitation) Albert Heyman, Atlanta, Ga.

*Abstract.* A high incidence of chancroid and lymphogranuloma venereum has been found in the general Negro hospital population of Atlanta. The incidence of these infections as compared to syphilis is about 40 per cent. A large-scale study of the clinical and laboratory aspects of chancroid and lymphogranuloma venereum was recently completed in our clinic. Biopsy and all other known diagnostic procedures such as cultures, smears, auto-inoculations, and skin test were employed in this investigation. Our histologic observations of chancroid and lymphogranuloma venereum were based upon cases proved either by culture of the Ducrey bacillus or isolation of the virus of lymphogranuloma venereum. Zenker's fixative and the phloxine methylene blue stain were used. The material from proved lymphogranuloma venereum was fixed in Regaud's fluid and stained with Giemsa's mixture.

It became apparent during this study that chancroid could be diagnosed by its histologic appearance alone. The lesion of chancroid shows a shallow ulcer with

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some purulent exudate beneath which two quite distinct zones of cellular infiltration are seen. The deep layer shows a dense infiltration of plasma cells and lymphocytes. There are numerous newly formed blood vessels which are arranged in palisading fashion. They show marked endothelial proliferation and, near the surface, degeneration of the vessel wall and thrombosis are commonly encountered. The tissue beneath the ulcer is cellular and consists chiefly of endothelial cells. These are found in all stages of proliferation and outnumber all other cells. The lack of appreciable proliferation of fibroblasts in the same area is striking and constitutes an important finding.

The primary lesion of lymphogranuloma venereum was studied in several proved cases. The histologic picture of this lesion is not well known, although the appearance of the lesions in lymph nodes has been described. The primary lesion is a shallow ulcer surrounded by an area of diffuse inflammatory cellular infiltration in which large mononuclear cells predominate. These form occasional small granulomatous foci which display central necrosis. Neutrophilic polymorphonuclear leukocytes fill the necrotic areas. These granulomata are similar to those seen in the lymph nodes. They are formed by proliferation of large mononuclear cells in the media and the adventitia of small blood vessels. This proliferation leads to the compression and disappearance of the vessel lumen, but there is no significant endothelial overgrowth. Solid collections of large mononuclear cells appear and become confluent. The occlusion of vessels leads to central necrosis and eventually to the formation of the ulcer.

It was found that the histologic picture of chancroid was sufficiently distinct to permit a reasonably certain diagnosis and to differentiate this infection from other venereal lesions. The diagnosis of chancroid by histologic examination was far more accurate than by smear. It was more reliable and a simpler method than culture which requires special technic and considerable experience. Among all diagnostic procedures, biopsy was the most efficient single method of diagnosis. Biopsy is not practical in the diagnosis of lymphogranuloma venereum. The primary lesion is evanescent and the removal of lymph nodes is not advisable. The histologic picture of the infection is, however, sufficiently distinct to permit the diagnosis. We have found that chancroid, lymphogranuloma venereum, syphilis, and granuloma inguinale as well as nonspecific genital lesions can be diagnosed by histologic examination with a high degree of accuracy. In our experience histologic examination of genital lesions offers more information than any other single diagnostic procedure.

DISSEMINATED GRANULOMA VENEREUM. John S. Howe and (by invitation) M. Markowitz, Richmond, Va.

*Abstract.* Granuloma venereum can no longer be considered a disease entirely localized to external genitalia and inguinal regions. Extragenital lesions occur in 6 per cent of cases, usually attributed to contact or auto-inoculation. Lesions of the cervix uteri have been described by several authors, and Pund and associates have described involvement of uterus, tubes and ovaries in 4 cases. Reports of more widely disseminated lesions have been few, and few necropsies have been reported.

We have observed at autopsy a case of extensive disseminated granuloma venereum in a 27-year-old Negress with rapid progression to a fatal outcome following pregnancy. The primary lesions were specific ulcers of cervix and vagina, followed by extension to the endometrium, myometrium, tubes and ovaries, rectum and bladder. There was extension by lymphatics to the iliac and lumbar lymph nodes and iliopsoas muscles bilaterally. There were also localized lesions of the cecum with involvement of the ileocolic lymph nodes, localized specific nodules in both kidneys, and a specific left pretibial ulcer, all probably due to retrograde lymphatic

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*Abstract.* Granuloma venereum can no longer be considered a disease entirely localized to external genitalia and inguinal regions. Extragenital lesions occur in 6 per cent of cases, usually attributed to contact or auto-inoculation. Lesions of the cervix uteri have been described by several authors, and Pund and associates have described involvement of uterus, tubes and ovaries in 4 cases. Reports of more widely disseminated lesions have been few, and few necropsies have been reported.

We have observed at autopsy a case of extensive disseminated granuloma venereum in a 27-year-old Negress with rapid progression to a fatal outcome following pregnancy. The primary lesions were specific ulcers of cervix and vagina, followed by extension to the endometrium, myometrium, tubes and ovaries, rectum and bladder. There was extension by lymphatics to the iliac and lumbar lymph nodes and iliopsoas muscles bilaterally. There were also localized lesions of the cecum with involvement of the ileocolic lymph nodes, localized specific nodules in both kidneys, and a specific left pretibial ulcer, all probably due to retrograde lymphatic



spread. Microscopically, all lesions showed foamy macrophages predominating, nearly all of which contained numerous intracellular Donovan bodies. Plasma cells were present in moderate numbers, and a few polymorphonuclear leukocytes were seen, some of them eosinophilic. Areas of fibrosis were present, particularly in the iliopsoas muscles. This appears to be the first confirmation of the reports of Pund and associates of granuloma venereum involving tubes and ovaries, and apparently records for the first time involvement of kidneys and iliopsoas muscles by granuloma venereum.

### Discussion

(Dr. Walter H. Sheldon, Atlanta, Ga.) We have seen many instances of granuloma inguinale (granuloma venereum) and have encountered several cases of this disease which showed systemic manifestations. Last year we reported a case of osteomyelitis of the tibia caused by granuloma inguinale. Since then we have seen a patient with granuloma inguinale involving the trachea and apparently the lungs. There was a tracheal fistula. Tissues taken for histologic study from the tracheal fistula and from a lower level of the trachea showed granuloma inguinale. The lungs showed extensive involvement on roentgenologic examination. At present we are studying a case in which there was extensive granuloma inguinale of the perineum 1 year ago. The perineal lesion had healed, but at autopsy multiple abscesses were found in the lungs and in one kidney.

It might be permissible here to emphasize another aspect of this disease. Extragenital lesions which are not necessarily systemic manifestations are quite frequent but unfortunately are not diagnosed, simply because they are not suspected. I have in mind the case of a man with a lesion on the lip, which is a common site of extragenital infection. For over 1 year this man had been treated vigorously for cancer. After the correct diagnosis was made, proper therapy caused healing of the lesion.

### DEVELOPMENT AND PATHOGNOMONIC EVALUATION OF THE STERNBERG-DOROTHY REED CELL. Fritz Levy, Elkins, W. Va.

*Abstract.* This is the first exhibition of a complete series of photomicrographs explaining the development of the Sternberg-Dorothy Reed cell. These plurivalent cells arise as typical blind alleys of development by pluripolar mitoses.

The exhibit shows: (1) photomicrographs of Sternberg-Dorothy Reed cells and comparable occurrences in other tissues; (2) diagrams of types of aberrant mitoses; (3) geometric comparison of sizes of nuclei in spheroidal and flat cells with valences 1:2:4.

The comparison with similar occurrences proves:

1. The Sternberg-Dorothy Reed cell develops principally in the way that we recently described for the development of the megakaryocytes of the bone marrow. We confirmed with new photomicrographs of megakaryocytes the findings formerly described with drawings by Heidenhain, myself, and Wuyts.

2. Isolated pluripolar mitoses occur in every kind of tissue when a cell division, especially a cleavage of the cytoplasm, is temporarily disturbed (by ether, colchicine, cold, some hydrocarbons, etc.).

3. Not uncommonly, pluripolar mitoses are found in tissue where a rapid cell division occurs in a small area. In normal human and animal tissue this is found exclusively (a) in the bone marrow, leading to the formation of megakaryocytes, (b) in spermatogenesis (best observed seasonally, e.g., in frogs), (c) in regenerating liver tissue.

4. Numerous pluripolar mitoses leading to the formation of plurivalent giant cells are found in rapidly growing tissue, as in many malignant tumors.

5. If the volumes of cells are in relation 8:16:32, then the radii of nuclei are, in spheroidal cells, 12:16:20; in flat cells, 16:23:32.

6. Nucleoli are especially flat parachromosomal formations. They, therefore, increase enormously in size in plurivalent cells.

7. The Sternberg-Dorothy Reed cells are more or less exactly 4, 8, 16, 32 valent cells of a tumor of flat cells, most probably a reticulo-endotheliosarcoma (Hodgkin's sarcoma, Karsner).

VISCERAL LESIONS OF ACUTE INFECTIOUS MONONUCLEOSIS. A REPORT OF TWO CASES WITH FATAL SPONTANEOUS RUPTURE OF THE SPLEEN. John H. Fisher, London, Ont.

*Abstract.* Heretofore, knowledge of the pathology of acute infectious mononucleosis has been derived chiefly from examination of the blood and bone marrow during life and from a study of excised lymph nodes. Extremely few opportunities have been afforded to study the disease process in the body as a whole at autopsy. Van Beek and Haex (*Acta med. Scandinav.*, 1943, 113, 125) have described lesions in the liver as seen in material obtained by aspiration from living patients. They claim that liver changes in mononucleosis have not been recorded previously.

Two cases are here reported in which death occurred from spontaneous rupture of the spleen. This is a very rare complication. Clinically and pathologically they were almost parallel cases. Both were young Canadian soldiers observed at autopsy in the Medical Services of the Canadian Army Overseas. Widespread accumulations of abnormal lymphocytes (mononucleosis cells) were found in most of the viscera. The lesions in the liver were similar to those described by Van Beek and Haex. Superficially, they reminded one of the liver in leukemia and also showed some resemblance to the liver lesions in the early stages of acute infectious hepatitis. The lymph nodes and spleen showed intense diffuse infiltration of their pulp with mononucleosis cells. The bone marrow was normal. The lesions of infectious mononucleosis as seen in these two cases are compared with those of acute lymphatic leukemia. Points of similarity between the disease process in acute infectious mononucleosis and that in acute infectious hepatitis are discussed.

#### Discussion

(Dr. R. H. Rigdon, Little Rock, Ark.) This spontaneous rupture of the spleen is very interesting. Sometimes we see a similar lesion in malaria. I have had opportunity to study the spleen in malaria and have found a proliferation of the cells in the splenic pulp projecting into the large sinuses. This produces mechanical blockage of the circulation. It has been suggested that this may be the basis for rupture of the spleen. It would be interesting to know whether or not you have observed such a proliferation of the cells in the splenic pulp in these cases of infectious mononucleosis. A similar process can be seen in cases of leukemia.

(Dr. Paul R. Cannon, Chicago, Ill.) Major Custer told me recently that they have seven cases of mononucleosis with rupture of the spleen at the Army Institute of Pathology, and suggested the possibility that in some instances palpation of the spleen might precipitate rupture. It should be noted, also, that in the older literature there are a number of probable cases of acute infectious mononucleosis with rupture of the spleen which have been reported as acute Hodgkin's disease.

(Dr. Horace K. Giffen, Youngstown, O.) Very recently our roentgenologist more or less incidentally made a roentgenogram on a case of acute infectious mononucleosis and found a picture which was reminiscent of the infiltration in psittacosis. Neither he nor I could find in the literature any suggestion of pulmonic lesions. I wonder if anybody else has x-ray findings on these cases.

(Dr. Kornel L. Terplan, Buffalo, N. Y.) I would like to ask the speaker whether

there were, in the two cases he has seen, any clinical symptoms pointing to meningeal involvement. In a case with rupture of the spleen which I had occasion to examine a few months ago, the clinical diagnosis leaned first strongly towards some peculiar basilar meningitis with involvement of the nerves of the ocular muscles. The patient recovered. The gross and histologic findings in the spleen, examined by me, were in line with the very few reports known from the literature.

(Dr. Fisher) In reply to Dr. Rigdon's question, I should say that in the literature there are many cases of spontaneous rupture of the spleen in malaria. In my two cases of mononucleosis the splenic sinuses contain numerous mononucleosis cells, and the splenic pulp is packed with similar cells which compress and narrow the sinuses. Concerning vascular obstruction, I cannot say more than that. However, in the two cases I am reporting I believe that there is no evidence that hemorrhage occurred within the splenic substance but rather that it commenced as subcapsular hemorrhage, gradually stripping off the capsule which finally ruptured, resulting in fatal intraperitoneal hemorrhage.

I should like to thank Dr. Cannon for bringing to my attention the seven cases that Dr. Custer is now collecting. I shall be much interested in seeing his report. In my first case the man died on a day on which medical ward rounds were held in the morning. No doubt his spleen was palpated several times by the attending staff. We considered this as a possible factor in promoting rupture of the spleen.

In reply to Dr. Giffen I would point out again that in mononucleosis there are pulmonary lesions, particularly in the perivascular and peribronchial connective tissue, but I do not know whether these lesions produce any demonstrable roentgenologic findings. I have seen no reference in the literature to such findings.

In reply to Dr. Terplan, no evidence of meningeal involvement was noted in either of my cases. Unfortunately, the brain was not examined in either case. However, meningeal involvement is fairly commonly encountered in mononucleosis and many such cases are now appearing in the literature.

**SYSTEMIC NONLIPOID RETICULO-ENDOTHELIAL GRANULOMA (LETTERER-SIWE'S TYPE): A PATHOLOGIC STUDY OF FOUR CASES.** Louisa E. Keasbey (by invitation) and William O. Russell, Los Angeles and Santa Barbara, Calif.

*Abstract.* This disease, observed in early childhood, is characterized by marked generalized hyperplasia of histiocytes, causing splenomegaly, generalized enlargement of lymph nodes, infiltrations in the skin, tumor-like replacement of bone, and a fatal anemia. The relation of the disease to infectious reticulosis, the infectious granulomas, and the diseases of disturbed lipid metabolism is discussed.

**DIFFERENTIATION OF LEUKEMIAS AND DISORDERS OF THE LYMPHATIC APPARATUS BY LEUKO-AGGLUTINATION.** Bernhard Steinberg and (by invitation) Ruth A. Martin, Toledo, O.

*Abstract.* Various types of circulating cells and some of the fixed tissue cells were differentiated by agglutination with specific antisera. Mature and immature lymphocytes and granulocytes were found to possess a cell specificity. On that basis, leuko-agglutination (clumping of circulating or fixed tissue cells by antibodies or similarly acting substances) was used to differentiate the various types of leukemia and lymphoblastoma. Normal peripheral leukocytes were antigenically similar to leukemia. Leukemic leukocytes were antigenically dissimilar from lymphosarcoma (reticulum cell type and malignant lymphocytoma, Ewing), and leukosarcoma. On the other hand, leukosarcoma (Sternberg) and lymphosarcoma were antigenically similar. Monocytic leukemia was distinct from the other forms of leukemia, but showed a group relationship to the lymphocyte.

GAUCHER'S DISEASE: HISTOCHEMICAL DEMONSTRATION OF KERASIN IN TISSUE.  
Joseph Kahn (by invitation) and Abraham R. Kantrowitz, Brooklyn, N. Y.

*Abstract.* Crystals of kersasin-like substances were demonstrated in tissues of Gaucher's disease. Frozen sections were completely dehydrated and defatted in successive changes of acetone followed by petroleic ether. Drops of quinoline were added to the slide. Mild heating, followed by cooling, permitted the crystallization of kersasin-like substances. A selenite plate of the first order was placed between the slide and the substage condenser of the polarizing Nicol's prisms. Against the salmon-red to pink background, Maltese crosses and, under higher magnification, hexagonal crystals with blue and yellow crossed segments became visible.

A brief résumé of the current status of the lipoid substances in Gaucher's disease was given.

FILARIASIS IN AMERICAN ARMED FORCES. William B. Wartman, Cleveland, O.

*Abstract.* This report is based on a study of material collected at the Army Institute of Pathology from cases of filariasis contracted during World War II. It consisted of 63 specimens taken for biopsy from military personnel and included 57 lymph nodes, 3 spermatic cords, and 3 epididymides. Accurate information about the epidemiology of the disease was available. About 90 per cent of patients were infected in the Samoan Islands, 4 per cent in the Solomon Islands, and the rest in the South Pacific area. One case was apparently infected in New Guinea. The average length of exposure in endemic areas was 11 months, with extremes of 1 to 30 months. The incubation period was between 5 and 15 months in most cases and the earliest proved case was 3 months.

Clinical findings consisted of genital lesions, acute lymphangitis of the extremities, especially the arms, lymphadenitis, and peculiar fugitive swellings. Constitutional manifestations were slight or absent; the attacks lasted a few days, and recurrences were the rule. Genital lesions included acute funiculitis, epididymitis, orchitis, inflammation of scrotal skin, and hydrocele. Lymphangitis was commonly manifested by raised, tender, red streaks and spread centrifugally. Lymphadenitis often preceded the lymphangitis. Microfilariae were absent from the blood in the great majority of cases, but were found in very small numbers on one occasion in each of 7 patients. Skin tests with *Dirofilaria immitis* antigen were positive in about 90 per cent of cases. Cultures of lymph nodes, blood, and hydrocele fluid were negative.

Tissue reactions consisted of acute and chronic granulomatous inflammation, proliferation of cells of the reticulo-endothelial system, and exudation of eosinophils. When adult worms were present, typical granulomas formed around them and there was striking proliferation of littoral cells and reticulum. In other portions of the node there was hyperplasia of lining cells of sinuses which often appeared as a tongue-like process growing into the distended channels. Eosinophils were abundant and sometimes there were "eosinophilic abscesses." Eventually, adult worms either disappeared or were encapsulated with collagenous connective tissue, or became calcified. Microfilariae were found in some specimens, but it was difficult to determine whether the accompanying tissue changes were due to microfilariae or to an allergic reaction to a nearby or distant adult worm.

#### Discussion

(Dr. Howard T. Karsner, Cleveland, O.) Dr. Wartman did not have time to refer to the diagnostic value of biopsy in filariasis. In this connection, the main question is whether, in those lymph nodes in which worms or microfilariae are not identified, the presence of granulomas and histo-eosinophilia is sufficient to justify a positive diagnosis.

(Dr. Wartman) In reply to Dr. Karsner's question I firmly believe that the only positive way of making a diagnosis, particularly in the occasional case, is by identification of the infecting organism, either adult worms, or the microfilariae. However, in cases in which there is known to be exposure to the disease, and in which there are clinical symptoms present, the finding of the other changes is confirmatory of the diagnosis.

THE SERODIAGNOSIS OF AMEBIASIS: EVALUATION OF THE CURRENTLY AVAILABLE ANTIGENS IN A QUANTITATIVELY STANDARDIZED COMPLEMENT-FIXATION TEST.

John F. Kent (by invitation) and Charles R. Rein, Washington, D.C.

*Abstract.* A complement-fixation test employing quantitative criteria in the standardization of reagents and test conditions has been applied in the evaluation of antigens for the serodiagnosis of amebiasis. Results obtained in experimental studies with serums from established cases of amebiasis, subjects with other diseases, and presumed normal persons indicate the advantages inherent in the quantitatively standardized procedure, and point out the limitations in sensitivity and specificity of the available antigens. Current studies directed toward improvement of the test are discussed.

ACUTE MALARIAL LESIONS PRODUCED IN CHICKS BY PLASMODIUM GALLINACEUM.

Lloyd R. Hershberger and G. Robert Coatney (by invitation), Bethesda, Md.

*Abstract.* Eight-day old chicks were inoculated intravenously with  $16 \times 10^6$  parasites. (Blood-induced infection.) The acute lesions in the major organs were then followed by tissue studies on chicks sacrificed on succeeding days. Malarial pigment deposits were present in the spleen, liver, bone marrow, kidney, heart, and brain, with variable disorganization caused thereby. Acute inflammatory lesions were noted in the spleen, lungs, and pancreas. The bone marrow showed moderate hyperplasia. Extramedullary hematopoiesis was seen in the liver, heart, and lungs. Slight fatty changes were present in the liver, heart, and kidneys. Exo-erythrocytic parasites were most frequently seen in the brain in the late stages of the acute infection.

*Discussion*

(Dr. R. H. Rigdon, Little Rock, Ark.) These pathologic changes described by Dr. Hershberger are very interesting. I would like to know whether his birds developed a severe anemia, because in following the pathologic changes in ducks with malaria we found that the characteristic process is a progressive anemia. We feel this is one of the important things in the mechanism of the pathologic changes and ultimate death.

I would like to ask if Dr. Hershberger looked for necrosis around the central veins of the liver. His comment with regard to edema of the heart muscle is interesting, due to the fact that we feel there is a circulatory failure based on the acute anemia in the duck. I wonder if that might not also occur in the chick. I wonder if there were any pathologic changes looked for specifically in the brain, since we have already described numerous degenerative changes which occur in acute malaria in the brain.

(Dr. Israel Davidsohn, Chicago, Ill.) How long was the interval between the inoculation and the death of the animal in those cases where there were extramedullary foci of hematopoiesis?

(Dr. Robert J. Parsons, Naval Hospital, Great Lakes, Ill.) I would like to ask if the exo-erythrocytic forms were not present in the other organs which Dr. Hershberger described. He mentioned them only in the brain. In my experience they occur in all organs of the body.

(Dr. Hershberger) In reply to Dr. Rigdon, we did not find any other lesion of the brain in these birds. Concerning anemia, the bone marrow showed evidence of hyperplasia in both the erythroid and myeloid series. Concerning foci of extramedullary hematopoiesis, we did not notice any difference in the survival of birds with or without extramedullary hematopoiesis. Almost uniformly there was extramedullary hematopoiesis in our birds. Concerning the exo-erythrocytic forms in other organs, our paper was intended to deal with the pathologic changes rather than the parasites in this disease.

(Dr. Davidsohn) The question was—how long was the interval between the inoculation and the death of the animals in those animals where there were extramedullary foci of hematopoiesis?

(Dr. Hershberger) The normal birds at this stage show extramedullary foci of hematopoiesis, and what we had was an increase; there was no significant difference between the chicks which had a lot of extramedullary hematopoiesis and those which did not.

RELATIVE ACTIVITY OF SULFONAMIDES AGAINST DYSENTERIC BACILLI AND THEIR TOXIC FILTRATES. F. J. Moore (by invitation) and J. Marmorston, Los Angeles, Calif.

*Abstract.* Growth-inhibiting concentrations of sulfathiazole, sulfadiazine, sulfaguanidine, and sulfasuxidine were determined *in vitro* for 38 strains of *Salmonella-Shigella* organisms. The first 2 were further examined against 83 strains. It was found that sulfathiazole was 2.5 times as active as sulfadiazine, 40 times as active as sulfaguanidine, and 100 times as active as whole sulfasuxidine (unhydrolyzed) against these bacteria on the average. Each of these four drugs was tested for *in vivo* activity against toxic Flexner filtrates administered intraperitoneally to mice or intravenously to rabbits. No protective or therapeutic action was noted. Conclusions were drawn as to the site requiring treatment in dysentery.

INTRACRANIAL NEOPLASMS PRODUCED IN DOGS BY METHYLCHOLANTHRENE. R. M. Mulligan and K. T. Neubuerger, Denver, Colo.

*Abstract.* The implantation of methylcholanthrene in 100 mg. doses in gelatin capsules beneath the dura over the right frontoparietal area of the cerebrum of 7 young male mongrel bulldogs resulted in the production of intracranial neoplasms in 3 dogs. These neoplasms were an extracerebral fibrosarcoma, a glioma with the histologic characteristics of a spongioblastoma polare, and a sarcomatous meningioma, observed after 378, 444, and 436 days respectively.

*Discussion*

(Dr. Percival Bailey, Chicago, Ill.) Some years ago one of my pupils placed capsules of methylcholanthrene in the cortex of several dogs. The carcinogen was inserted directly into the cortex. It is very easy to produce tumors with carcinogens from the meninges of dogs. All of these animals developed convulsions after 6 to 8 months or so without the carcinogenic agent being anywhere near the motor cortex. Besides that, there was no reaction on the part of the neuro-epithelial tissue, and I would have to be convinced that the tumor reported here as a spongioblastoma was of neuro-ectodermal origin. I say that because in other cases I have not been able to convince myself that the tumors reported were really of neuro-epithelial origin. Dogs develop sarcomas of the brain spontaneously and rather frequently. Moreover, it has proved difficult to produce gliomas in the brains of animals, except in those of one particular strain of mice. I feel, therefore, that conclusive evidence is necessary before this tumor is labelled spongioblastoma polare.

(Dr. Mulligan) We studied these tumors with a number of different stains. With the hematoxylin and eosin stain there was a pronounced difference between the fibrosarcoma and the spongioblastoma. The nuclei in the fibrosarcoma had rather small basophilic nucleoli, and on the average they were much shorter than in the tumor which we think is a spongioblastoma. In the spongioblastoma the nuclei were much longer and there were eosinophilic nucleoli. There was a distinct difference in the chromatin pattern as well. I also mentioned the absence of any reticular fibrils in the glioma with the reticulum stain, whereas the fibrosarcoma contained many such fibrils. If you do not mind, I think that Dr. Neubuerger would be happy to send you slides to see what you make of these tumors yourself.

(Dr. Bailey) I would be glad to examine them.

(Dr. Howard T. Karsner, Cleveland, O.) This should be incorporated into the record.

(Dr. Bailey subsequently examined sections of these tumors and stated: "I believe all three of the tumors to be of connective tissue nature and that none of them is neuro-epithelial.")

STUDIES ON CAPILLARY PERMEABILITY AS AFFECTED BY ANOXEMIA. Howard C. Hopps, Oklahoma City, Okla. (by invitation) and Julian H. Lewis, Chicago, Ill.

*Abstract.* The minimum latent period for anaphylactic shock in guinea-pigs following passive sensitization is presumed to be an indication of the time necessary for antibodies to escape from the blood stream and into the tissues. This minimum latent period was not shortened as the result of acute anoxemia brought about by subjecting passively sensitized guinea-pigs to low oxygen tensions. Therefore, anoxemia, under these conditions, does not facilitate the passage of antibody globulin through vascular endothelium.

Studies on the rate of disappearance of T-1824 from the blood stream indicate that acute anoxia acts to *decrease* slightly the normal rate of disappearance of this dye. As an explanation of this phenomenon it is suggested that (a) anoxia, under these conditions, does not facilitate the passage of albumin-T-1824 complex through vascular endothelium and (b) anoxia acts to inhibit the mechanism by which T-1824 normally leaves the blood stream.

Significant alterations in the quantity of serum protein following acute anoxia produced under the conditions of the experiment were not observed.

PARENCHYMATOUS DEGENERATION RELATED TO ANOXIA. Virgil H. Moon, Philadelphia, Pa.

*Abstract.* Acute parenchymatous degeneration is commonly attributed to toxic effects, as in severe infections, poisoning, and intoxications, both endogenous and exogenous. But it develops also under other conditions, particularly when anoxia is a factor in causing death. In observations on the pathology of shock, parenchymatous degeneration was found regularly. This was noted in clinical cases of shock from trauma, surgery, burns, and in experimental shock produced by various means. Degenerative changes were especially marked in the kidneys and liver, less so in the myocardium. In cases of trauma and burns, toxic substances absorbed from damaged tissue might be suggested as causing parenchymatous degeneration. However, there are instances in which no toxic effect seems possible. Acute parenchymatous degeneration was found after death by coronary occlusion, nitrous oxide anesthesia, suffocation, heat stroke, anaphylaxis, and after shock from high-altitude aviation. Anoxia seems to be the common denominator in the various conditions mentioned and should be recognized among the causes for parenchymatous degeneration.

*Discussion*

(Dr. Joseph Tannenbergh, Batavia, N. Y.) I would like to ask whether Dr. Moon has seen, in these cases, focal necroses in the liver and eventually in the heart. During 1936 to 1938 I studied, in quite extensive experiments on rabbits, what I then called anoxic shock. Short periods of intensive anoxia were produced under simultaneous elimination of carbon dioxide in order to produce conditions which were purely due to the anoxia. In these rabbits I saw quite a good many focal necroses within the liver and within the heart, the skeletal muscles, and the brain. I have not seen, as far as I can remember at this time, anything that looked like focal necrosis in the kidneys.

(Dr. Howard T. Karsner, Cleveland, O.) In connection with functional disturbances, I wish to refer to the brief report by E. E. Selkurt which appeared in The Clinical Bulletin of the School of Medicine of Western Reserve University and Its Associated Hospitals, 1945, 9, 87-94. This was based on production of complete renal ischemia and on hemorrhagic shock. In both, there was widespread nephrosis in the kidneys (dogs) which were examined microscopically, evidently the result of local anoxia. The results indicated that the renal tubular excretory mechanism is impaired and that there is almost complete resorption of tubular fluid and its contained substances, the injured tubular cells losing their normal selectivity.

(Dr. C. V. Weller, Ann Arbor, Mich.) If Dr. Moon found that the cases with traumatic shock, which he used, were complicated by massive pulmonary fat embolism, that fact would explain the anoxemia. I do not think he made it clear whether that possibility was investigated.

(Dr. Norbert Enzer, Milwaukee, Wis.) I would like to state also for the record and as a tribute to a great member of this society, Dr. Oscar Schultz, that more than 20 years ago he spoke of the kidney of low oxygen. Time and again, in the laboratories, he called attention to precisely the changes reported here today. He commented also on the fact that kidneys removed at operation, particularly those in which the operative procedure was rather difficult and prolonged, frequently showed the pathologic changes in the renal tubules which have been referred to here. This subject has an important practical significance for those engaged in medico-legal pathology, in differentiating death from shock due to accident as against death from nontraumatic causes, where recognition of these changes may be effective in establishing the cause of death.

(Dr. Alex B. Ragins, Chicago, Ill.) I would like to ask in the case of the high-altitude deaths whether or not the changes in the liver were similar to those I have observed in cases of airmen who, returning from their high-altitude run, reported being well over the telephone communications system and 15 minutes later were found to be dead in the plane. On microscopic examination their livers showed peculiar vacuolization in the liver cells. There were neither fat nor glycogen-containing vacuoles. I wonder if Dr. Moon noticed that in his cases, and what they might be?

(Dr. Moon) I am deeply gratified by the interest that has been shown in this presentation. I can only comment briefly on the suggestions that have been made.

There were very marked changes in the liver in these cases as well; sometimes it was in the nature of focal necrosis; in other instances scattered groups of cells or individual cells were necrotic. I found focal necrosis rarely in the renal cortex, but rather necrosis of individual cells or groups of cells, as shown in the photomicrographs, in various parts of the tubules.

Pulmonary fat embolism was possible in cases of severe trauma. Unfortunately we did not have opportunity to test for fat in the pulmonary capillaries because the cases came from the Army Institute of Pathology and only stained sections were available. In other instances there appeared no opportunity for pulmonary fat embolism; deaths from coronary occlusion, nitrous oxide anesthesia, anaphylaxis, or from other causes probably were not complicated by fat embolism.



In high-altitude shock, of which 5 cases were examined, there was very marked degeneration of the liver as well as of the kidney. Had there been time, it would have been interesting to show corresponding sections of the liver and the kidney in each case, showing parenchymatous degeneration ranging in degree to necrosis of the cells.

#### THE INFLUENCE OF AGE AND SPECIES ON THE NEPHROTOXIC ACTION OF DL-SERINE.

Robert P. Morehead and (by invitation) W. D. Poe, J. O. Williams, and M. E. Lazenby, Winston-Salem, N. C.

*Abstract.* In previous experiments it has been shown that *dl*-serine exerts a pronounced nephrotoxic action in white albino rats weighing 100 gm. When animals of this type are placed on an experimental diet supplemented with *dl*-serine and deficient in the B vitamins, a high mortality follows, and the changes in the kidneys are more severe than in animals receiving the amino acid and maintained on a stock diet considered adequate. Addition of the B vitamins to the experimental diet reduces the mortality greatly but does not alter the renal lesions.

Young guinea-pigs and rabbits were placed on an experimental diet deficient in the B vitamins, and *dl*-serine was administered daily. A high mortality resulted, but no lesions were demonstrable in the kidneys. A group of mature mice were maintained on the deficient diet and given *dl*-serine daily. No deaths occurred in this group, nor were lesions demonstrable in the kidneys. Young mice are at present being employed under the same experimental conditions.

#### Discussion

(Dr. E. T. Bell, Minneapolis, Minn.) I would like to point out that something may be learned about how a toxic substance acts upon the kidney if one ureter is ligated. Ligation of the ureter suppresses glomerular filtration on that side. For example, if a ureter is ligated 24 hours or more before injection of bichloride of mercury or uranium nitrate, there is no injury to the hydronephrotic or obstructed kidney. On the other hand, sucrose produces great changes in the obstructed kidney, as in the normal. Evidently, substances like bichloride and uranium nitrate are concentrated in the tubule and produce their changes in that way.

(Dr. Paul R. Cannon, Chicago, Ill.) I would like to ask if this effect of *dl*-serine was produced by any of the other amino acids.

(Dr. Morehead) Many amino acids have been found to be nephrotoxic, particularly when injected. This action appears, however, to be confined for the most part to very young, growing animals. The changes in the kidney following the administration of these substances have more closely resembled those of acute cortical necrosis than the changes resulting from *dl*-serine. I might add further that in recent experiments we have found that *l*-serine is not nephrotoxic.

#### INFLUENCE OF EXPERIMENTAL RENAL DAMAGE ON HISTOCHEMICALLY DEMONSTRABLE LIPASE ACTIVITY IN THE RAT. COMPARISON WITH PHOSPHATASE ACTIVITY. Max Wachstein, Middletown, N. Y.

*Abstract.* The lipase activity of the kidneys of different animals was examined with the microtechnic of Gomori (*Proc. Soc. Exper. Biol. & Med.*, 1945, 58, 362-364) with some modifications. Preparations stained for alkaline phosphatase were also made. The rat was found to have a very constant lipase activity restricted to the cytoplasm of the cells composing the proximal convoluted tubules. After poisoning with uranium nitrate as well as mercury bichloride, the enzyme was not inactivated in necrotic and destroyed cells. However, there was a marked decrease

of its amount in the cells of regenerating and atrophic tubules. In rats in which severe hemorrhagic necrosis was produced by means of dietary choline deficiency, there was depletion of the enzyme in the necrotic cells in the acute stage and marked deficiency of the enzyme in the kidneys of animals surviving the acute damage. After ligation of the ureter, marked depletion of the enzyme occurred wherever the hydronephrotic changes were prominent. Alkaline phosphatase activity showed quite similar changes under the experimental conditions mentioned above. Wherever lipase activity in the convoluted tubules was diminished, this was found to be true also for the phosphatase activity in the cytoplasm of cells in this location. The significance of lipase and phosphatase activity in the convoluted tubules is not definitely clarified. Cellular damage, however, influences both enzymes in a very similar manner.

### *Discussion*

(Dr. G. Gomori, Chicago, Ill.) I would like to ask whether you have demonstrated lipase in man, either in normal or pathologic conditions. I have about twenty-five sections of normal and abnormal human kidneys and I have never found even traces of lipase.

(Dr. Wachstein) Neither in surgically removed kidneys nor in very fresh post-mortem material could lipase activity be found in tissue sections. However, in view of the fact that this enzymatic activity depends to a large extent on the substrate used and that lipase is found by biochemical methods in human kidneys, it is hoped that by applying a suitable substrate, lipase activity will also be demonstrated in tissue section.

(Dr. H. Edward MacMahon, Boston, Mass.) I would like to ask if one sees the return of this material during repair as rapidly as one sees its histologic disappearance in the early stages of disease.

(Dr. Wachstein) Wherever cells show loss of enzymatic activity they are also different in sections stained with hematoxylin and eosin. These morphologically changed cells did not show a return of enzymatic activity during the time the experiments were carried out.

### EXPERIMENTAL STREPTOCOCCAL INFECTIONS OF THE CHORIOALLANTOIC MEMBRANE OF THE EMBRYONIC CHICK. Noble P. Sherwood, H. R. Wahl, Catherine Colglazier (by invitation), and Tom R. Hamilton, Lawrence, Kan.

*Abstract.* This paper contains a comparison of the pathologic changes and host-parasite relationships of 24 strains of hemolytic streptococci representing the Lancefield groups A to K. The results reveal a striking difference in invasive property, necrosis of ectoderm and mesoderm, ability to spread within the mesoderm and to multiply within phagocytic cells of the mesoderm. The 6 strains from cases of endocarditis, while more invasive than many other strains, exhibited wide differences in ability to spread and find host cells within the mesoderm. No correlation between virulence and hemolysins for chicken cells was observed.

### MENINGOCOCCIC PURPURA AND THE SHWARTZMAN PHENOMENON: AN EXPERIMENTAL STUDY. B. Black-Schaffer and (by invitation) G. P. Kerby and T. G. Hiebert, Durham, N. C.

*Abstract.* The Shwartzman phenomenon (largely by means of bacterial filtrates) has been the method of demonstrating the presence of a purpurogenic factor in many strains of meningococci. The time required to produce the phenomenon closely parallels the interval between the first symptoms and the appearance of the cutaneous purpura in many instances of clinical purpuric meningococcemia. The

unexplained selectivity of the human disease finds its analogue in the widely differing response of individual rabbits to the same dose of Shwartzman substances.

Because of these observations, and in order to more closely simulate human purpuric meningococcemia, the investigation here reported was undertaken. Living washed organisms recovered from an instance of purpuric meningococcemia were used throughout. It was discovered that after two washings with normal saline solution the organisms possessed the ability to prepare (intradermal inoculation) the skin of rabbits and elicit (intravenous inoculation) the phenomenon without the agency of the usual potent filtrates. The same properties were demonstrated by heat-killed, washed meningococci. In contrast to Shwartzman's experience, the washed organisms were more potent than the homologous supernatant fluid.

It is concluded that purpuric meningococcemia suggests a spontaneously occurring Shwartzman phenomenon; the organisms present in the purpuric lesions may be the counterpart of those intradermally inoculated into the rabbit. The meningococcemia, then, probably represents the provocative factor, just as do the organisms intravenously administered in the experiment.

### *Discussion*

(Dr. John R. Schenken, Omaha, Neb.) How does the essayist account for the appearance of purpura in cases within an hour or two after the onset of the disease? We reported a case where the child was perfectly well at 4 o'clock in the afternoon and dead at 7:30 P.M., having developed purpura in that period of time.

(Dr. Joseph Tannenber, Batavia, N. Y.) I would like to ask whether there were hemorrhagic infarctions of the adrenals also, in the experiments reported, as is the case frequently in patients dying of the Waterhouse-Friderichsen syndrome.

(Dr. Black-Schaffer) In response to the first question, it seems to me that in those cases which I have observed the patients are usually ill with a respiratory infection a day or two before the onset of the disease. The organisms are capable of getting into the skin during the course of the prodromal syndrome. In animals, all we need is a matter of 2 hours, once the organisms invade the blood stream, to produce the phenomenon. After that time the lesions manifest themselves as small cyanotic areas which then become purpuric and eventually develop a necrotic center; so that, while I cannot give you an authoritative answer, I think that the experiment appears capable of explaining the appearance of purpuric spots only a few hours after the apparent onset of the disease, which corresponds to the meningococcemia.

In reply to the second question, I hesitate to suggest that the adrenal hemorrhages are solely explicable on the basis of the Shwartzman phenomenon. They may occur in experimental animals after one intravenous inoculation of the substance, and I think that this may represent something similar to the hemorrhage-producing ability of the Shwartzman substance in tumors, where it is possible to destroy some forms by one intravenous inoculation. We would expect to find more patients with the massive type of adrenal hemorrhage were it produced by the mechanism of the usual Shwartzman phenomenon.

**HISTOPATHOLOGIC STUDY OF ANAPHYLACTIC SHOCK IN IDENTICAL TWINS.** Jacob Werne and (by invitation) Irene Garrow, New York, N. Y.

*Abstract.* Histologic study in identical male twins, 10 months of age, dying of delayed anaphylactic shock (16 and 20 hours after their second immunizing injection of diphtheria toxoid and pertussis antigen) disclosed visceral changes, of which the most prominent feature was vascular injury. The lesions encountered were similar in both cases, with minor variations. There was thickening of small

arterial walls, with extreme luminal narrowing; endothelial degeneration and early proliferation; exudation of fluid into vessel walls and about capillaries; necrosis of occasional arteries, with young thrombi; widespread capillovenous engorgement, with focal hemorrhages; and acute degeneration of parenchymal cells in brain, heart, lung, liver, adrenal, and lymphatic tissue. Tissue eosinophilia was most marked in bone marrow and thymus; conspicuous intravascular eosinophils were noted in some cerebral and pulmonary vessels, and in hepatic sinusoids. Narrowing of some bronchial and bowel lumina was evident. The follicles of the spleen, lymph nodes, and intestines showed marked phagocytic activity. Large amounts of ingested nuclear debris were present at their centers. Pulmonary emphysema was not present, indicating that the small amount of bronchoconstriction noted histologically was unrelated to the fatal outcome. Engorgement of the liver was extreme and was the most conspicuous gross finding in one twin; in the other, focal pulmonary hemorrhages, which on microscopic examination showed early bronchopneumonic consolidation, constituted the only distinctive gross finding.

The post-mortem investigation excluded contamination of the biological therapeutic agents, and of detectable disease. Attempts at passive transfer, using post-mortem serum, were unsuccessful. There was a history of angioneurotic edema in the father. The widespread morphologic appearances are interpreted as reflecting the known effect of the antigen-antibody reaction upon the smooth muscle and vascular endothelium of the anaphylactic body. In the cases presented the pathologic picture is uncomplicated by associated therapy or concurrent illness. It contributes further evidence of the basic rôle of vascular injury in the hypersensitive state.

#### *Discussion*

(Dr. Edmund Mayer, Stamford, Conn.) I wonder what emphasis has been placed in your investigation on the fact that these infants happened to be twins. What was the evidence that they were identical twins? Do you think that the distribution and quality of the lesions were more similar in those twins than one would expect to find in two infants of the same age who died from the same serum, but were not identical twins?

(Dr. Werne) The fact that the twins reacted in an identical manner to the second injection of a product which, so far as we have been able to determine, has not resulted in a fatality, is in conformity with our knowledge of the biologic similarities exhibited by identical twins. There were minor variations among the lesions encountered, such as more extensive hepatic congestion in Gary, and the presence of foci of hemorrhagic bronchopneumonia in Donald. The diffuse vascular lesions with their associated parenchymal degenerative changes were alike in both. Incidentally, the physician who gave the immunizing injection was the one who delivered the twins. He stated that they had a common placenta.

(Dr. B. Black-Schaffer, Durham, N. C.) Was the Prausnitz-Küstner reaction carried out to demonstrate sensitivity?

(Dr. Werne) Attempts at post-mortem transfer were made using four volunteers among the laboratory staff. This was not successful. Dr. Jules Freund of the Bureau of Laboratories of the New York City Health Department, who made exhaustive studies on the product in order to exclude primary toxicity and contamination, attempted previous transfer, using post-mortem serum, to guinea-pigs, also without success. According to the available literature, there is no constant relationship between the clinical response and the demonstrable reagin. Walzer reported several cases in which, at the height of the anaphylactic shock, previous transfer was unsuccessful; following recovery he was successful in demonstrating reagin. We are familiar, of course, with the success of Lund and Hunt in demonstrating the Prausnitz-Küstner reaction in post-mortem serum from their case of instantaneous anaphylactic death.

THE NONPORTAL DISTRIBUTION OF THE TRABECULAE IN DIETARY CIRRHOSIS OF RATS AND CARBON TETRACHLORIDE CIRRHOSIS OF RATS AND GUINEA-PIGS.\*  
L. L. Ashburn, K. M. Endicott, F. S. Daft (by invitation), and R. D. Lillie, Bethesda, Md.

*Abstract.* The purpose of this study was to obtain further information on the relationship of the trabeculae to the portal and hepatic veins in certain experimental cirrhoses. Albino rats at weaning were placed on a low-protein, choline-deficient diet, and killed after 50 to 150 days. The livers were injected through the portal or hepatic veins with a charcoal gelatin mass to effectively mark these structures in the microscopic preparation. Histologic study showed that the fatty deposition, ceroid accumulation, and fibrous trabeculation primarily followed and connected hepatic veins. In livers showing marked alteration, the trabeculae sometimes included, coursed by, or abutted on large portal areas. However, even in these livers the portal areas comparable in level to the centro-lobular veins were not primarily related to the trabeculae. In other experiments, cirrhosis in rats and guinea-pigs was produced by the repeated subcutaneous administration of carbon tetrachloride. The livers were injected with the charcoal gelatin mass and studied histologically. The connective tissue trabeculae occurring in these livers were primarily related to hepatic veins and showed essentially the same distribution as that seen in dietary cirrhosis.

HEMOPOIESIS IN FOLIC ACID AND RIBOFLAVIN DEFICIENCY. K. M. Endicott and (by invitation) A. Kornberg and M. Ott, Bethesda, Md.

*Abstract.* Folic acid deficiency occurs in rats given a purified diet containing sulfasuxidine. This results in pancytopenia and especially granulocytopenia. Similar blood dyscrasias develop in rats fed a diet deficient in riboflavin. Comparative quantitative studies of hemopoiesis in these two deficiencies were made. In both deficiencies there is a progressive depletion of all types of cells of the granulocytic and megakaryocytic series. The spleen becomes atrophic with small inactive follicles and no hemopoiesis. The thymus involutes and lymph nodes become atrophic. The lymphopenia, granulocytopenia, and anemia of folic acid deficiency are spectacularly remedied by daily oral doses of crystalline folic acid. This is accompanied by a marked regeneration of bone marrow, splenic hemopoiesis, and increased size and activity of the lymphoid apparatus. The lymphopenia and granulocytopenia of riboflavin deficiency respond somewhat more slowly to crystalline folic acid therapy and this slower response is also noted in the bone marrow, spleen, and lymphoid apparatus. The anemia of riboflavin deficiency responds to riboflavin therapy but not to folic acid therapy.

BLINDNESS IN DUCKS ACCOMPANYING HYPOGLYCEMIA.\* R. H. Rigdon and (by invitation) D. E. Fletcher, Little Rock, Ark.

*Abstract.* Ducks given large amounts of insulin develop a marked hypoglycemia and, accompanying this decrease in sugar, the birds lose their sight. This loss of vision is a temporary change which persists as long as the sugar level is abnormally low. Sight returns when the sugar returns to normal amounts. This change apparently results from a disturbance in cerebral glycolysis. Acute degenerative changes occur in the optic nerve and the brain of these birds.

*Discussion*

(Dr. Joseph Tannenbergh, Batavia, N. Y.) Six years ago I presented before the meeting of this Association a paper with the title, "Anatomical Changes Produced by Short Periods of Anoxia (Anoxic Shock). The Effect of Frequent Repetitions

\* This article will appear in a subsequent issue of *The American Journal of Pathology*.

and Combinations with Insulin" (*Am. J. Path.*, 1940, 16, 656-657). I went the other way around and studied first what I called anoxic shock. In this condition intensive anoxia was applied until the animals were seized by convulsions or the respiration stopped. In these experiments we found an absolutely identical symptomatology in both types, anoxic and insulin shock, and the same anatomic changes that were demonstrated today. Various degenerative lesions were produced throughout the brain. Since these shocks were frequently repeated, on some animals 20 to 30 times; we found many transitional stages between fresh and older lesions. Particularly well demonstrable were lesions in the cerebellum, affecting the Purkinje cells, many of which were degenerated. I do not know whether these rabbits became blind, since they were kept in cages, but some might have been.

(Dr. Kornel L. Terplan, Buffalo, N. Y.) I would like to ask Dr. Rigdon whether any attempt was made to weigh the brains of the ducks which died in the acute stage of shock. The most impressive findings in the cases of fatal insulin shock in men, as reported 9 years ago at the Chicago meeting (*Am. J. Path.*, 1937, 13, 664-666), was a considerable increase in the weight of the brain from true swelling. Obviously the sensitivity of the various parts of the brain in ducks is different from that in the human brain. In the human material examined by me the most severe changes were in the cortex. In contrast, there was comparatively little damage in the brain stem. These marked changes in the cortex were identical with changes seen in severe cases of swelling of the brain from various causes (including oleum *Chenopodium* poisoning, avertin anesthesia with cessation of breathing, and severe burns). We explained them on the basis of anoxia rather than as in any way specific for hypoglycemia.

(Dr. Tannenber) The physiologic basis which explains why anoxic and insulin shock have the same symptomatology is this: According to the work of Himwich, the brain cells can live only on carbohydrate and on nothing else. Therefore, for their metabolism two things are required: blood sugar and oxygen. The same effect should be obtained when either of them is deficient.

(Dr. Rigdon) In answer to Dr. Terplan's question about the weight of the brain in these ducks, I can only state that we did not weigh any of them. It is of interest to observe these lesions in the optic tracts, brain stem, and cerebellum of ducks while similar lesions occur in the cortex in man. Of course the duck does not have a cerebral cortex.

#### READ BY TITLE

OBSERVATIONS ON THE CULTIVATION OF BACTERIUM TULARENSE IN EMBRYONATED EGGS. Lewis L. Coriell, Capt., M.C. (by invitation), Cora M. Downs, Gifford B. Pinchot, Lt., U.S.N.R. (by invitation), Elizabeth Smadel, and (by invitation) Alice Klauber, Lt., U.S.N.R., Lawrence, Kan.

*Abstract.* Studies using a measured number of virulent *B. tularensis* as inoculum in embryonated eggs showed that the organisms grew more abundantly in the cells of the yolk sac than in the chorioallantoic membrane or in the embryo. The organisms grew equally well in duck eggs. Strains of *B. tularensis* of lowered virulence grew less vigorously than virulent strains, whereas the completely avirulent strain 38 did not survive or multiply in embryonated eggs. *B. tularensis* also grew well in eggs in which the embryo was killed at the time of inoculation. Serial passage of virulent strains did not alter their virulence for mice or the chick embryo. The structural changes produced by *B. tularensis* in the embryonated egg are described.

GENERALIZED BOECK'S SARCOIDOSIS WITH THROMBOCYTOPENIC PURPURA. Norbert Enzer, Milwaukee, Wis.

*Abstract.* The patient was a man, 32 years of age, who was observed for a period of 3 years. His first complaints were considered suggestive of peptic ulcer, although

at that time hydrochloric acid was absent from the stomach and roentgenograms failed to reveal an ulcer. He continued to have intermittent abdominal pain, for which an appendectomy was finally performed. The appendix showed only mild inflammation. Roentgenograms of the chest about 2 years before death disclosed fibrosis of both upper lobes. A tuberculin test was negative repeatedly and gastric washings were likewise negative for tubercle bacilli. About 8 months before death he developed a severe anemia, thrombocytopenia, leukopenia, and purpura. Examination of the bone marrow showed normal hyperplasia. All efforts to relieve the hematopoietic situation failed and finally, with the purpura becoming more severe, a splenectomy was performed. The spleen was enlarged and nodular and filled with lesions typical of Boeck's sarcoid. Death occurred from postoperative hemorrhage. Autopsy disclosed sarcoidosis of the lungs and lymph nodes. No lesions of Boeck's sarcoid were found in the bones or bone marrow.

**EXTENSIVE DESTRUCTION OF THE BRAIN IN ECLAMPSIA.** Herman Josephy and Edwin F. Hirsch, Chicago, Ill.

*Abstract.* Eclamptic convulsions in a young woman were followed by vegetative existence until death 3 months later. The brain had extensive regions of destruction in various stages of organization. There are only a few descriptions of this condition in the literature.

**BILATERAL ACUTE HEMORRHAGIC NECROSIS OF THE ADRENALS IN A YOUNG CHILD.**  
(A CASE OF WATERHOUSE-FRIDERICHSEN SYNDROME.) Joseph Tannenberg, Batavia, N. Y.

*Abstract.* In a rural area of western New York about 5 cases of meningococcal infections, most of which were cases of meningitis, were seen annually in the past 5 years. In 3 cases a meningococcal septicemia was present. Two of them showed multiple petechiae in the skin. All recovered under treatment with sulfadiazine and, later, penicillin. In a neighboring county we have seen recently a case of a child of 5 years who, on the second day of what appeared to be a slight cold, died in convulsions on the way to the hospital. The autopsy showed nothing but a slight hyperemia of the leptomeninges! Only histologically was it possible to make the diagnosis of a fresh meningococcal meningitis, with recovery of meningococci. There was no involvement of the adrenals. Quite in contrast to these cases stands a case that presented the typical Waterhouse-Friderichsen syndrome. A child in a good state of nutrition and previously in good health suddenly was taken ill with fever up to 102°F. No changes in the lungs were shown roentgenologically. During the first day of his illness multiple large petechiae developed in the skin. The child died 2 hours following the first administration of 20,000 units of penicillin. At autopsy a bilateral complete hemorrhagic infarction of the adrenals was found. Blood taken from the right ventricle remained, on culture, sterile for meningococci. One tonsil cultured yielded a hemolytic staphylococcus which gave a positive coagulase test. Histologically, there was no evidence of meningitis; practically no other changes were found except partial necrosis of lymph follicles in the spleen and large secondary follicles in the tonsils. Here a number of cocci were seen within phagocytic cells. These cocci were single and Gram-positive. No diplococci were recovered. A few days before the child was taken sick the mother had tonsillitis which, a week after the death of the child, led to the development of otitis media. It is held that bilateral hemorrhagic infarction of the adrenals may occur in rare instances of septicemia, particularly in those caused by the meningococcus. However, it is not held that this anatomic picture is absolutely diagnostic for meningococcal infections, particularly when it is considered that even in the largest series of 19 cases, which were reported from a single institution, it had been possible only in 6 cases to recover meningococci from the blood and organs of the stricken person.

# THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XXII

JULY, 1946

NUMBER 4

## NECROTIZING ARTERIAL LESIONS RESEMBLING THOSE OF PERIARTERITIS NODOSA AND FOCAL VISCERAL NECROSIS FOLLOWING ADMINISTRATION OF SULFATHIAZOLE

### REPORT OF A CASE \*

LOUIS LICHTENSTEIN, M.D., and LEON J. FOX, M.D.

*(From the Laboratory Division, Hospital for Joint Diseases, New York 35, N.Y.)*

With the advent of the sulfonamides there came reports of many and varied clinical manifestations of toxicity and sensitivity that so often follow the introduction of a new drug. These various reactions have been reported in a voluminously growing literature,<sup>1-3</sup> and range in nature from mild febrile reactions<sup>4</sup> to marked skin sensitivity,<sup>5</sup> focal visceral necrosis, and, as most recently reported by Rich,<sup>6-9</sup> vascular lesions resembling periarteritis nodosa.

Hageman and Blake,<sup>10</sup> as early as 1937, recognized the possibility of reactions to the sulfonamides of an allergic nature and commented on the similarity of the clinical picture to the manifestations of serum sickness. In 1939, Erskine<sup>11</sup> suggested that this reaction might be mediated by the conjunction of sulfonamides with serum proteins, and, indeed, Schönholzer,<sup>12</sup> Wedum,<sup>13</sup> Davis,<sup>14</sup> and others subsequently demonstrated the linkage of sulfonamides to plasma protein in experimental studies. It appears that of all the sulfonamides in current use, sulfathiazole is perhaps the chief offender in this respect.

As for the anatomic expression of these untoward reactions, French and Weller,<sup>15</sup> in 1942, called attention to the rather frequent finding of interstitial myocardial lesions, rich in eosinophils, in subjects known to have received one or another of the sulfonamides within 30 days of their death. Similar focal lesions were also observed by them in the lung, liver, and kidney, and comparable lesions were reproduced in mice and rats. Shortly thereafter, Lederer and Rosenblatt<sup>16</sup> reported on their findings in 4 cases treated with sulfathiazole, in which numerous areas of focal necrosis were noted microscopically in practically all viscera. About the same time, Merkel and Crawford<sup>17</sup> reported 4 cases with similar lesions in which sulfathiazole was also implicated.

Rich,<sup>6,7</sup> in 1942, observed vascular lesions resembling periarteritis

\* Received for publication, June 26, 1945.



nodosa in two subjects who had been treated with sulfathiazole. Since then, he has seen a number of similar cases.<sup>18</sup> These vascular lesions were associated with the presence also of widespread focal necrosis such as Lederer and Rosenblatt<sup>16</sup> and others had previously described. The vascular lesions were not unlike those Rich had observed in fatal cases of serum sickness, and he considered them to have developed on the basis of hypersensitivity reactions. Duff,<sup>19</sup> Black-Schaffer,<sup>2</sup> and Moore<sup>20</sup> have likewise observed arterial lesions in autopsied subjects who had received sulfonamides. Murphy, Kuzma, Polley, and Grill<sup>21</sup> also noted similar lesions in the kidney in cases with predominantly renal involvement.

Rich<sup>9</sup> has recently commented on the possibility that hypersensitivity to chemicals other than sulfonamides may also result in vascular lesions. He specifically cited an autopsy in which lesions resembling those of periarteritis nodosa appeared in a patient who had exhibited manifestations of hypersensitivity to iodine, which was administered for hyperthyroidism. In this connection, we might mention that we have also observed necrotizing arterial lesions, in association with a purpuric skin eruption, in a subject who had received moderate doses of phenobarbital over a prolonged period. It is of interest to note, furthermore, that Marine and Baumann<sup>22</sup> have recently described vascular lesions like those of periarteritis nodosa in 3 of 100 or more rats which had been fed thiouracil.

As for the sulfonamides, in general most of the sensitivity phenomena have followed their oral administration, their topical application, or their administration by the parenteral route. It has been recognized that similar sensitivity reactions can occur also after the local introduction of the sulfonamides into clean surgical wounds; but, to our knowledge, no fatal reactions have been described under these circumstances. We had occasion recently to observe a case in which the patient showed evidences of sensitivity reaction to sulfathiazole which had been introduced into a clean surgical wound but in which, despite this reaction, sulfathiazole was subsequently given by mouth, on the assumption that the wound had become infected. This patient died and was autopsied. It is of considerable interest that one of the manifestations of hypersensitivity was the presence of widespread vascular lesions resembling those of periarteritis nodosa, lesions such as Rich<sup>6-9</sup> has described.

#### REPORT OF CASE

*Clinical History.* The patient was an obese Negress, 61 years of age, who had had an open reduction for a subcoracoid dislocation of the right shoulder of 4 months' duration. Prior to closure of the wound, sulfathiazole was dusted into it, the amount thus introduced being 5 gm. or less. As far as is known, the patient had been in

good health prior to her injury and had had neither hypertension nor renal disease.

The postoperative course until the ninth day was uneventful. Then the patient had a chill, with an elevation of temperature to 103° F. At this time, it was noted that a vesicular skin eruption was present, small lesions being scattered over the trunk and extremities. The patient stated that these had been present for several days.

The wound was examined, but no evidence of infection was found. Cultures were taken of the wound, the vesicular skin lesions, and the blood. These remained sterile. Meanwhile, the patient was started on sulfathiazole, 1 gm. every 4 hours, on the premise that she had a wound infection, possibly associated with septicemia. Sulfathiazole was administered for 6 days, when it was discontinued at the advice of the dermatologic consultant, but not until the patient had received 31.5 gm. Penicillin was substituted without apparent benefit. After a few days, it was suspected that the patient's difficulty might be due to sulfathiazole reaction rather than infection, but by this time irreparable renal damage had been done, as evidenced by albuminuria, red blood cells in the urine, oliguria, steadily increasing azotemia (the nonprotein nitrogen mounting to 207 mg.), manifestations of acidosis, and finally the full syndrome of uremia. It is interesting to note, also, that the free cholesterol of the serum was as high as 75 per cent, indicating severe hepatic damage, and that the serum bilirubin was increased fivefold, suggesting increased destruction of red blood cells. The patient expired about 3 weeks after the operative intervention.

### *Post-Mortem Examination*

The body was that of a large, obese, elderly Negress in rigor mortis. There was a recent surgical incision, approximately 10 inches in length, over the anterolateral aspect of the right shoulder and upper arm. It was partly closed by skin sutures and appeared to be healing. The shoulder and upper arm region appeared edematous and presented a brawny induration, but no evidence of infection. The skin presented clusters of dried, encrusted papules (apparently representing a healing stage of the vesicular lesions noted clinically). In addition, the skin also showed some evidence of scaling, but no hemorrhagic or bullous lesions were observed. The conjunctivae were intensely congested, and on the right showed edema as well. There was no peripheral edema, jaundice, or enlargement of the superficial lymph nodes.

The panniculus adiposus measured as much as 6 cm. in thickness, the fat being bright yellow. The peritoneal surfaces were smooth and glistening. There was a small quantity of cloudy yellowish fluid in both pleural cavities. The visceral pleura, especially over the lower lobes, appeared dull and in places was coated by fresh fibrinous exudate. Smear of this fibrin revealed the presence of pus cells, but no bacteria were found. The corresponding pulmonary parenchyma was firm and section revealed a patchy bronchopneumonia. The cut surfaces of the lungs, otherwise, were dark red and congested. The trachea and bronchi were filled with pinkish froth. There was no gross evidence of tuberculosis in the lungs or hilar lymph nodes.

The parietal and visceral layers of the pericardium were adherent. The heart was slightly enlarged in consequence of dilatation of its chambers. The myocardium was soft and yellow-brown. The valves showed no evidence of endocarditis. The coronary arteries and their branches appeared somewhat thickened, but were nowhere narrowed or occluded.

The liver was slightly enlarged, rather firm, pale, and presented a finely granular surface in places. The lobular architecture on section was not clearly outlined. The gallbladder was filled with numerous, small, faceted whitish stones, which on section presented the structure of mixed cholesterol-pigment gallstones.

The spleen was somewhat enlarged, but did not extend below the costal cage. The splenic pulp was very soft, although not diffuent, and the gray pulp was very prominent.

The adrenal glands presented nothing remarkable.

The pancreas was rather large, pale gray-yellow, and unusually firm.

The kidneys, embedded in a large mass of congested and indurated perirenal fat, were soft and friable. The cortical surfaces were relatively smooth, and showed no apparent hemorrhagic lesions grossly. On section, the kidneys presented evidence of severe parenchymatous degeneration. The glomeruli stood out as unusually prominent grayish flecks. There were no hemorrhages noted in the renal pelves, nor was any gravel or crystalline material observed in them. The ureters were not unusual. The urinary bladder was slightly dilated and presented a small diverticular out-pouching on its posterior wall. No concretions were found in the bladder, and the mucosa presented nothing remarkable, except for a single petechial hemorrhage.

Examination of the genital tract revealed atresia of the cervical canal, thick, yellow-brown, pus-like fluid in the endometrial cavity, and a slightly dilated and sealed right fallopian tube.

Examination of the gastrointestinal tract revealed nothing noteworthy. The mesenteric lymph nodes were not appreciably enlarged.

We were not permitted to examine the brain and spinal cord.

Portions of two ribs, a slice of the body of the sternum, and part of the 4th lumbar vertebral body were taken for routine histologic examination.

#### Microscopic Examination

Microscopic sections revealed rather unusual lesions which had not been anticipated from the gross findings. These were essentially of two types: first, focal necrosis in the liver, lung, myocardium, spleen, gallbladder, and pancreas; and second, necrotizing arterial lesions like those of periarteritis nodosa in the kidneys, liver, spleen, gallbladder, adrenal glands, uterus, fallopian tubes, and elsewhere.

The focal visceral lesions, as noted, were widespread. They consisted essentially of rather small and fairly localized collections of inflammatory cells, associated with necrosis at the site of the lesion. The inflammatory cells were small mononuclear cells and polymorphonuclear leukocytes, including some that appeared to be eosinophils. These necrotic foci were most numerous in the heart (Fig. 7) and pancreas (Fig. 9), but could be found readily in many other viscera. In the liver (Figs. 5 and 6), for instance, numerous foci were observed both in the lobules and within periportal fields, where some of them had apparently broken into the lumina of radicles of the portal vein.

The vascular lesions also had a wide distribution (Figs. 1 to 5). They found their fullest expression in the kidneys (Figs. 1 to 3), but they were occasionally encountered in the spleen and gallbladder and, as noted, there was a scattering of them also in many other viscera. For the most part, it was the smaller arteries and arterioles that were affected, which perhaps would explain why none of the lesions were detectable grossly. Furthermore, virtually all of the lesions appeared to be fresh or acute, which is in keeping with the brief duration of illness in this case. Characteristically, there was observed at the site of these vascular lesions more or less complete fibrinoid necrosis of the wall of the artery, including its media. In hematoxylin and eosin preparations this was reflected by a homogeneous, bright eosin-staining, smudgy appearance. In many of the arterial lesions streaks of the same fibrin-like material extended into the perivascular exudate. The necrotic wall was infiltrated in places by polymorphonuclear leukocytes. Radiating out from the wall of the vessel were oval or spindle-shaped cells apparently of histiocytic nature. Interspersed with and surrounding these cells there was a perivascular inflammatory exudate of pleomorphic nature, including lymphocytes, plasma cells, mononuclear macrophages, and numerous polymorphonuclear leukocytes including some eosinophils. In some of the affected arteries the endothelial lining cells were found to be unusually large and swollen. In general, the appearance of these necrotizing arterial lesions was such that we could not distinguish them from the classical lesions of periarteritis nodosa.

*Anatomical Diagnoses.* Status (3 weeks) after open reduction of (4 months' old) subcoracoid dislocation of right shoulder, and the administration of sulfathiazole: necrotizing arterial lesions resembling those of periarteritis nodosa in kidneys, liver, spleen, gallbladder, adrenal glands, uterus, and fallopian tube (developing apparently on the basis of hypersensitivity to sulfathiazole); widespread foci of visceral necrosis; terminal azotemia; acute fibrinous pleuritis and patchy bronchopneumonia (bilateral); brawny induration of extracapsular soft tissues of right shoulder region; severe secondary anemia (hemato-

logically); severe parenchymatous degeneration of kidneys; enlargement of spleen; chronic cholecystitis and cholelithiasis; old pericardial adhesions; pleural adhesions (left); scarring of media of aorta (probably on old luetic basis); obesity.

### SUMMARY AND CONCLUSIONS

A description is given of the changes observed at autopsy in a case of what appeared to be hypersensitivity to sulfathiazole, which had been introduced into a clean surgical wound made in the course of an open reduction of a subcoracoid dislocation. On the ninth postoperative day, the subject developed a chill, elevation of temperature to 103° F., and a vesicular skin eruption. The significance of these manifestations of sulfonamide hypersensitivity was not appreciated, and sulfathiazole was again administered on the mistaken premise that there was a wound infection, possibly associated with septicemia. The subject died about 3 weeks after the operative intervention with manifestations of uremia, evidence of severe hepatic damage, and also hyperbilirubinemia. The significant pathologic changes were the following: (1) necrotizing arterial lesions resembling those of periarteritis nodosa in the kidneys, liver, spleen, gallbladder, adrenal glands, uterus, and fallopian tube; (2) focal visceral necrosis in the myocardium, liver, lungs, spleen, gallbladder, pancreas, and elsewhere; (3) severe parenchymatous degeneration of the liver and kidneys; (4) acute fibrinous pleuritis and patchy bronchopneumonia (bilateral).

The changes found in this case have been discussed in relation to those in comparable cases in the literature. This case appears to be the first recorded instance of a fatal hypersensitivity reaction in which the initial sensitization was brought about by the introduction of sulfonamide into a clean surgical wound.

Failure to recognize the clinical manifestations of hypersensitivity to the sulfonamides (fever, chill, a skin eruption of hemorrhagic, vesicular, or bullous character, and failing renal function on or shortly after the seventh day following the administration of sulfonamides) may have serious consequences. Sometimes, as in the case presented, these manifestations may be misinterpreted as indications of infection calling for the administration of sulfonamides with renewed vigor, the effect of which may be likened to pouring gasoline on a fire.

Because sulfathiazole appears to be the principal offender in respect to hypersensitivity reactions, the indiscriminate use of this drug for minor infections or for prophylaxis seems ill advised. For enteral or parenteral use, sulfadiazine, for instance, is preferable, and for wound implantation, sulfanilamide.

Attention is directed to the fact that the administration of chemicals other than the sulfonamides (e.g., iodine, phenobarbital, and thiouracil) also may be followed occasionally by the appearance of periarteritis nodosa-like lesions.

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## DESCRIPTION OF PLATES

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### PLATE 128

FIG. 1. A representative field of the kidney. In the center of the field there is a necrotic artery surrounded by a collar of inflammatory cells. In the lower right corner there is another perivascular collection of cells about a small artery, which in part is still preserved. The tubules are spread apart by edema, and the interstitial connective tissue is infiltrated by inflammatory cells.  $\times 65$ .

FIG. 2. Higher magnification of the renal artery illustrated in Figure 1. The entire wall of the artery, including its media, is necrotic and in hematoxylin and eosin stain gave a homogeneous bright red, fibrinoid appearance. Streaks of this fibrin-like material extend out into the perivascular zone. Surrounding the arterial wall is a mantle of histiocytes or monocytes, and beyond this a zone of closely packed inflammatory cells may be seen, including mononuclear macrophages, lymphocytes, plasma cells, and polymorphonuclear leukocytes. Some of the leukocytes appeared to be eosinophils.  $\times 200$ .

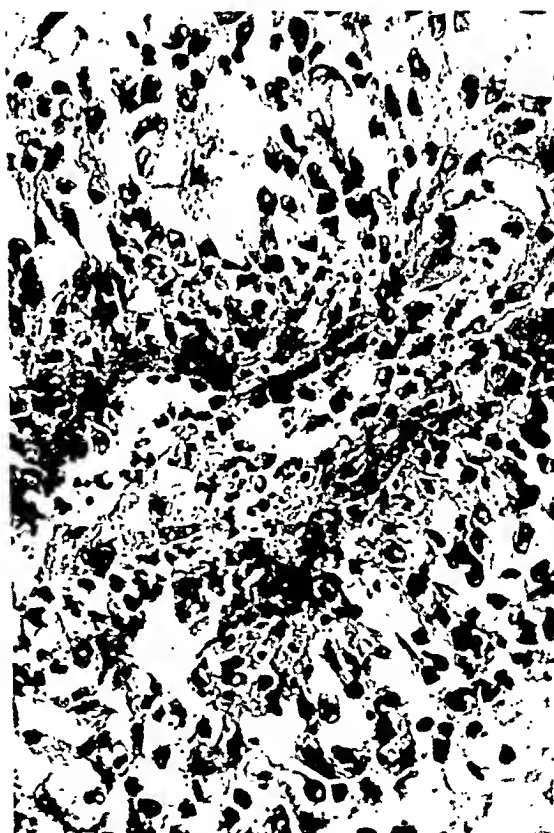
FIG. 3. Another interlobular artery in the kidney. There is fibrinoid necrosis and infiltration of the wall of the vessel by polymorphonuclear leukocytes. Radiating out from the necrotic arterial wall are oval or spindle-shaped histiocytic cells.  $\times 375$ .



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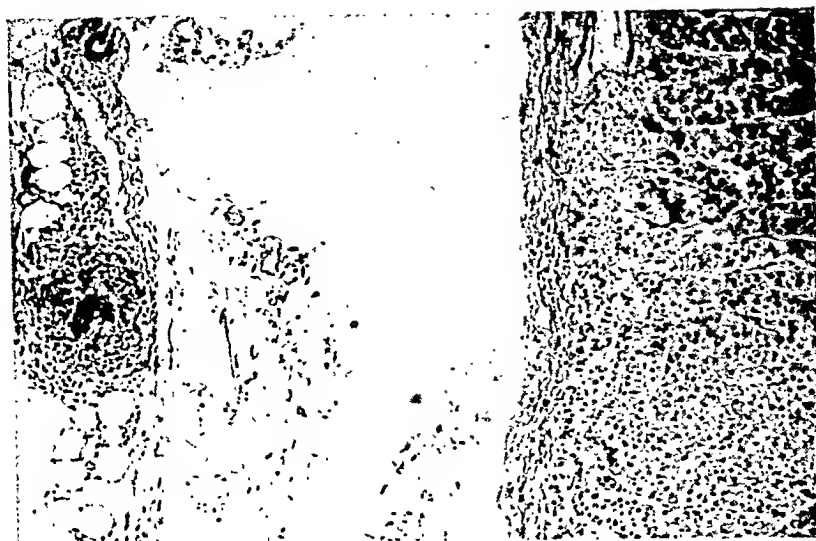
Lichtenstein and Fox

Arterial Lesions Following Sulfathiazole



PLATE 129

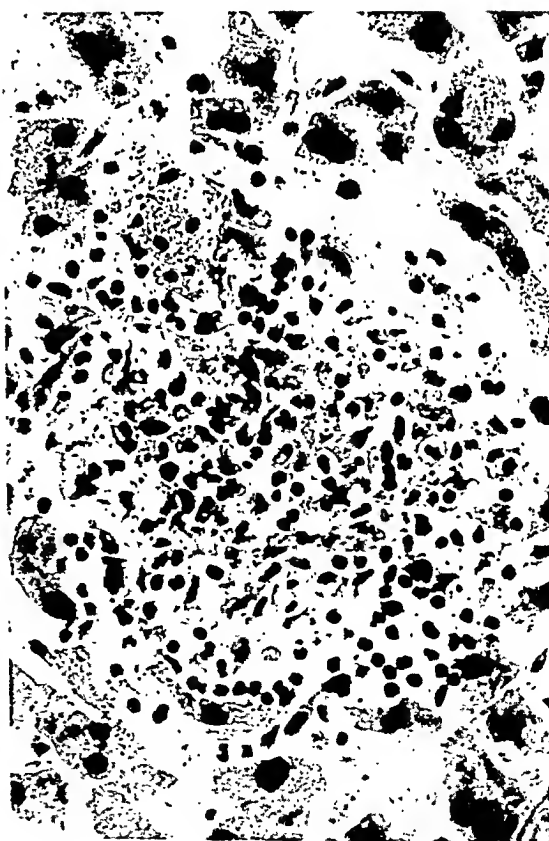
- FIG. 4. A necrotic small artery in the fatty tissue around the adrenal gland.  $\times 65$ .
- FIG. 5. A periportal field in the liver. Below the center there is an affected arterial branch surrounded by a zone of inflammatory cells. There are also collections of inflammatory cells elsewhere in the periportal field. With hematoxylin and eosin stain one could observe fibrinoid necrosis of the vessel wall, and also that an appreciable number of the leukocytes were eosinophils.  $\times 75$ .
- FIG. 6. A focus of necrosis and cellular infiltration within a liver lobule. The inflammatory cells are of varied character and represent lymphocytes, plasma cells, mononuclear macrophages, and polymorphonuclear leukocytes, including a considerable number of eosinophils.  $\times 350$ .



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6

Lichtenstein and Fox

Arterial Lesions Following Sulfathiazole

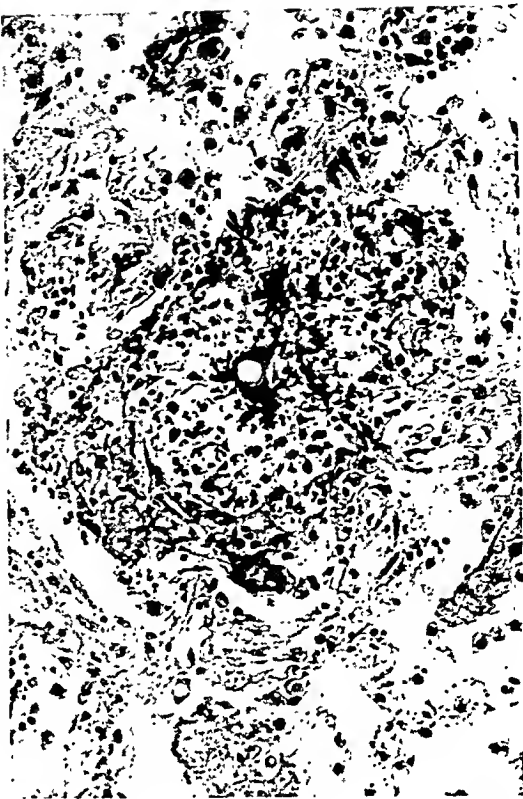
PLATE 130

- FIG. 7. Focal necrosis within the myocardium. At higher magnification it was evident that a majority of the inflammatory cells were polymorphonuclear leukocytes.  $\times 250$ .
- FIG. 8. A field in the lung. The dark-staining material within the alveolar walls and alveolar spaces resembles fibrin in the hematoxylin and eosin preparation, and is interspersed with macrophages and polymorphonuclear leukocytes.  $\times 200$ .
- FIG. 9. A representative field in the pancreas. In the center of the field there is a small roundish focus of necrosis and cellular infiltration. At higher magnification it was observed that most of the cells within this focus were polymorphonuclear leukocytes.  $\times 200$ .
- FIG. 10. Sulfa-crystals within a renal tubule. There were comparatively few such crystal present. The more fully calcified masses no longer retained this characteristic structure.  $\times 850$ .

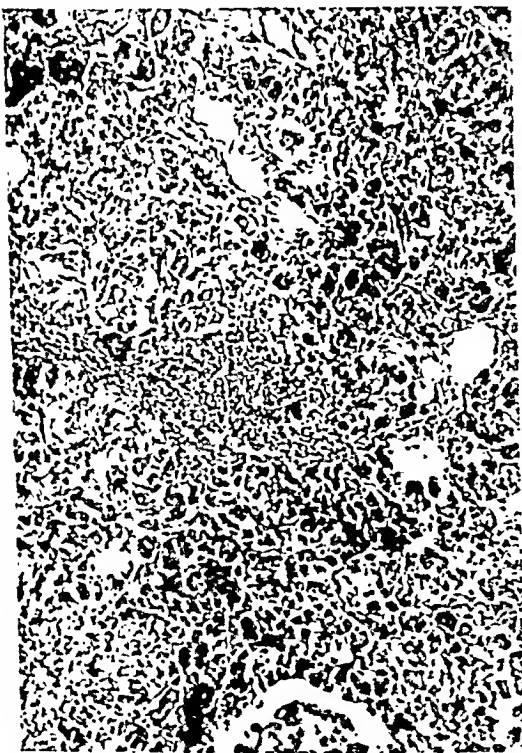
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# HYPERSENSITIVITY IN THE PATHOGENESIS OF THE HISTOPATHOLOGIC CHANGES ASSOCIATED WITH SULFONAMIDE CHEMOTHERAPY \*

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An earlier report on the effects of the sulfonamide drugs in tissues was published in collaboration with C. V. Weller in 1942.<sup>1</sup> At that time few reports of this character had been released, but subsequently there has been an increasing number in the literature. As each new sulfonamide derivative was introduced, the hope that it would prove to be less toxic than preceding ones was revived; however, after it had been used for a time, investigation proved that comparable tissue changes took place in one or several organs of the body.

The material utilized in this report includes 76 fatal cases investigated at the Army Institute of Pathology from 1937, when neoprontosil was first used therapeutically in Army hospitals, until 1943, when a variety of sulfonamides were being employed.

Skin taken for biopsy from 2 other cases was included in the material, making a total of 78 cases. This series is approximately one-sixth of more than 500 cases in which one or another type of lesion had resulted from the administration of sulfonamides, but complicating disease factors caused the other cases to be eliminated. All cases were excluded in which sulfonamide drugs had been given in the treatment of any of the following conditions: septicemia, confirmed by blood culture; rheumatic fever; cardiovascular disease, including coronary thrombosis; poliomyelitis; scrub typhus or other proved viral or rickettsial infection; trichinosis; diphtheria; scarlet fever; typhoid fever; or miliary tuberculosis. However, in the presence of many of these complicating diseases, cellular infiltrates were observed which were identical with those regarded as the characteristic sulfonamide effect.

The sulfonamide drugs which had been administered to the patients in this series included neoprontosil, sulfanilamide, sulfathiazole, sulfapyridine, sulfaguanidine, sulfadiazine, and the sodium salts of sulfathiazole, sulfapyridine, and sulfadiazine. Various combinations of these drugs were given to 24 patients. In four instances "sulfonamide therapy" was reported without reference to the specific drug employed (Table I).

The total dosage of sulfonamide drugs received by the patients during the terminal illness varied from 8 to 340 gm., over periods ranging

\* Received for publication, September 30, 1945.

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from several days to 6 weeks. No case was included in the series in which death occurred later than 1 month after cessation of sulfonamide therapy. In all but 2 cases chemotherapy had been instituted at least 72 hours before death, but in these the drug had been given intravenously in moderately high dosage.

*Clinical Data.* Sulfonamide drugs were administered to 30 patients who were admitted to the hospital with a diagnosis of nasopharyngitis. Lobar pneumonia due to type I pneumococcus developed in 3 of these

TABLE I

*Sulfonamide Drugs Administered to Patients Included in the Reported Series\**

Drug	No. of cases
Neoprontosil	1
Sulfanilamide	6
Sulfathiazole	22
Na sulfathiazole	3
Sulfapyridine	1
Na sulfapyridine	1
Sulfadiazine	11
Na sulfadiazine	4
Sulfaguanidine	1
Combinations	24
"Sulfonamide therapy"	4

\* Two biopsy cases included.

cases, due to type III pneumococcus in one, and in the remaining 26 there was terminal bronchopneumonia. Eleven of the 76 patients were being treated for gonorrhea and the remaining 35 for a variety of clinical conditions including fracture, perforated appendix, duodenal ulcer, and otitis media.

*Induced Sensitivity.* In approximately one-half of the cases reported in this series there was clinical evidence of some reaction

denoting sensitivity to the sulfonamide drugs. Table II lists these clinical complications which suggested sensitivity.

In 14 cases, more than one course of treatment with sulfonamides had been resorted to during the terminal illness of the patient. The first therapeutic course did not produce severe complications in all cases; apparently, in some the degree of sensitivity increased with repeated courses of the drugs. Rash, fever, chills, nausea, vomiting, cyanosis, or leukopenia were the usual signs of unfavorable reaction with the first course of sulfonamide therapy; hypoplastic anemia, icterus, anuria, dermatitis, or combinations of such grave complications, with a subsequent course of treatment. The exact length of treatment was not stated in the brief clinical summaries submitted with the protocols in most instances, so that the relationship of duration of therapy to clinical complication could not be calculated. Patients with hypoplastic or hemolytic anemia, dermatitis or anuria died following varying periods of therapy, but no definite relationship between induced sensitivity and duration of therapy, dosage, or a specific sulfonamide drug was apparent in cases completely reported. Multiple courses of sulfonamide therapy separated by intervals varying from hours to weeks appeared to result in acquired sensitivity.

## HISTOPATHOLOGY

Histopathologic lesions were found most frequently in the heart, liver, and kidney. Sections of intestine, gallbladder, urinary bladder, prostate, testis, lymph node, bone marrow, skin, skeletal muscle, and meninges were only occasionally represented in the material examined so that the corrected incidence of pathologic changes in these organs probably would be somewhat higher. The normal cellularity of organs such as the spleen, lymph nodes, and bone marrow contributed to the difficulty of establishing an increase in cell content, but the acidophilic cellular reactions to the sulfonamide drugs were obvious in these organs.

*Heart*

The heart lesions indicative of sulfonamide intoxication were those described by French and Weller<sup>1</sup> in experimental and clinical material and confirmed by Frist<sup>2</sup> and Flynn.<sup>3</sup> The characteristic cell found in the lesions in

the heart and in all other organs examined was the acidophilic histiocyte. This cell, with variable numbers of other mononuclear and polymorphonuclear cells, both acidophilic and neutrophilic, was present in paravascular foci, or diffusely distributed between the cardiac muscle fibers, in the subepicardial areolar tissues, and beneath the endocardium. Necrosis was neither constant nor prominent in the tissues in which these cellular infiltrates were found, but did occur in the more severe cases (Figs. 1 and 2).

Distinctive vascular involvement (Figs. 3 and 4), such as that described by Rich<sup>4</sup> and confirmed in a personal review of this material by him, was noted in many of the hearts examined. In 16 cases vascular lesions were seen in organs other than the heart. The lesions consisted of endothelial edema and proliferation, fibrinoid necrosis of the vessel wall, and endarteritis and periarteritis with acidophilic histiocytes and eosinophils predominating in the inflammatory cellular reaction. The infiltrate in older lesions contained fewer eosinophils and relatively more histiocytes.

In addition to the inflammatory cellular reaction, epicardial, myocardial, or subendocardial hemorrhages, petechial or diffuse in character, were seen in the sections. Characteristic cellular infiltrates were

TABLE II

*Clinical Complications in the Reported Cases Associated with the Administration of Sulfonamide Drugs*

Clinical complications	No. of cases
Dermatitis	19
Anuria	9
Icterus	8
Aplastic anemia	7
Fever and/or chill	6
Eosinophilia in peripheral blood	2
Hemolytic anemia	2
Leukopenia	2



present throughout the walls of capillaries and venules adjacent to or in the hemorrhagic areas. In a few instances early fibroblastic proliferation was associated with inflammatory infiltrates; however, experimental reproduction of this change will be required before its significance in relation to the sulfonamide drugs can be evaluated.

Extensive focal calcification of the myocardium was observed in one case and calcification of isolated muscle fibrils in another; in neither was there evidence of vascular occlusion or other cardiac disease. A minimal acidophilic cellular infiltration in the adjacent myocardium was attributed to the sulfonamide drugs (Fig. 5). The significance of calcification of the myocardium was not apparent and its interpretation must await further experimentation. Calcification of the myocardium in rats fed sulfonamides, as reported by Endicott, Kornberg, and Daft,<sup>5</sup> may be a comparable lesion.

### *Liver*

Lesions in the liver believed to result from the administration of sulfonamide drugs included infiltrations containing acidophilic histiocytes and neutrophils with varying degrees of focal necrosis or micro-abscess formation (Fig. 6). The presence of dense cellular infiltrations containing characteristic acidophilic cells, in the absence of other known cause for such collections of inflammatory cells (*i.e.*, cholelithiasis, choledocholithiasis, or other detectable biliary tract disease) was interpreted as evidence of reaction to sulfonamide drugs during the terminal illness.

In the liver as in the heart there was a minor degree of cellular infiltration in the wall of an occasional central vein. Hemorrhage was not noted in the liver.

### *Kidney*

Renal lesions of many kinds were encountered in approximately one-half of the cases reviewed. Those believed to have been caused by the action of sulfonamides included *interstitial nephritis*, in which characteristic acidophilic mononuclear and polymorphonuclear cells were conspicuous components (Fig. 7); crystal formation (Fig. 8), often associated with calcium deposition, or with accumulations of bluish staining material both intratubularly and extratubularly; and vascular fibrinoid necrosis, thrombosis, subpelvic hemorrhages, and cellular infiltrates. Both focal necrosis and cellular infiltrates were seen in sections showing interstitial nephritis.

Tubular lesions were for the most part limited to the distal portions of the nephron, including the ascending limb of Henle's loop and the distal convoluted and collecting tubules, in which necrosis of the tubu-

lar epithelium and regeneration of the lining cells were seen. The lumina were plugged and the tubules distended with hemoglobin casts, crystals, crystals combined with calcium salts, erythrocytes, leukocytes, and amorphous protein precipitates. Some tubules showed necrosis of the lining with rupture of the walls and escape of crystals into the adjacent interstitial tissues. Glomerular and proximal tubular lesions were less frequent but glomerular vascular lesions were striking when present.

Subpelvic hemorrhages were conspicuous if ureteral and pelvic catheterization had been employed before death in an attempt to remove sulfonamide crystals. Hemorrhages and characteristic cellular infiltrates beneath the pelvic epithelium were also seen when there had been no surgical intervention. As in the heart, these hemorrhages and infiltrates were believed to be attributable to the sulfonamide drugs.

### *Skin*

Sections of skin were included in only 2 of the fatal cases and were submitted for biopsy in 2 others. Inasmuch as a rash was reported in 18 cases, the skin changes must be regarded as significant. In the 4 specimens examined the characteristic acidophilic cells were present. The lesions, both clinically and histopathologically, were like those seen in erythema multiforme with vesiculation (Fig. 9) and in erythema nodosum (Fig. 10). The cellular infiltrates were mainly paravascular with some diffusion of cells in the more severe cases. Vascular involvement was not a salient feature.

### *Testis*

Acidophilic histiocytes were striking in the sections of testis included in 4 cases in the series (Fig. 11). Because testicular tissue was submitted in so few cases, the true incidence of lesions in the testes could only be estimated. Although the acidophilic histiocytes were distributed in the supporting tissues of the testis, there was no possibility of confusion with Leydig cells. No necrosis or hemorrhage was present in the sections of testis examined.

### *Spleen, Lymph Nodes, and Bone Marrow*

Specimens of spleen were received in 64 of the 76 cases studied. In 7, miliary foci of necrosis, similar to those reported by Lederer and Rosenblatt,<sup>6</sup> and Merkel and Crawford,<sup>7</sup> were encountered in the red pulp. These foci of necrosis were associated with acidophilic histiocytes and neutrophilic leukocytes (Fig. 12).

Lymph node lesions were present in 3 cases. As in the spleen and

bone marrow, microscopic foci of necrosis were seen in association with acidophilic histiocytes, granular leukocytes, and hyperplastic reticulum cells (Fig. 13).

Bone marrow changes were present in 5 cases in the form of miliary areas of focal necrosis and infiltrations of acidophilic histiocytes and polymorphonuclear cells.

### *Lung*

Pulmonary lesions were the most difficult to interpret because of the bacterial inflammatory reaction usually present. However, in 4 instances definite intra-alveolar and interalveolar, peribronchiolar, peribronchial, and perivascular infiltrations of acidophilic histiocytes and polymorphonuclear cells were present. Vascular fibrinoid necrosis, capillary thrombosis, fibrinoid plaques in the alveoli, and interstitial cellular foci were noted in the lungs in addition to hemorrhage.

In 2 cases with acidophilic cellular infiltrate, massive pulmonary hemorrhage by diapedesis was present. In one case miliary necrotic foci were present in the lungs as well as in the liver, spleen, lymph nodes, and bone marrow. As already stated, any case in which there was miliary tuberculosis, tularemia, typhoid fever, or other known cause of focal necrosis was excluded from the series.

### *Other Organs*

Foci of eosinophilic histiocytes have been found in practically all organs of the body, including all parts of the gastrointestinal tract, gallbladder (Fig. 14), urinary bladder, skeletal muscle, and meninges. Tissue from these organs was not routinely submitted in the 76 cases reported and, as a result, the lesions were encountered too infrequently to be significant statistically. They are important in that they showed the characteristic cellular infiltrates.

### DISCUSSION

Histopathologic changes in the kidneys have received more attention in the literature than those of other organs. As noted by Prien, Crabtree, and Frondel,<sup>8</sup> Climenko and Wright,<sup>9</sup> Antopol, Lehr, Churg, and Sprinz,<sup>10</sup> and Hellwig and Reed,<sup>11</sup> renal tubular damage was usually confined to the distal portion of the nephron, particularly the distal convoluted and collecting tubules. Hemoglobin casts were an outstanding feature in many instances and were indistinguishable, unless associated with crystals of acetylated sulfonamides, from the hemoglobin-laden tubules seen following transfusion incompatibility, hepatitis, blast or crush injury, or death from burns. Interstitial cellular infiltrations,

containing acidophilic histiocytes and neutrophils, and subpelvic hemorrhages were components of the renal lesions noted in cases receiving the sulfonamide drugs.

It is remarkable that so little significance has been attached to the possible clinical effect of the sulfonamide drugs on the heart. Except for the reference of Scheinberg and Ingle<sup>12</sup> to myocardosis in a patient who recovered following treatment with sulfanilamide, no direct reference to the effects of the drugs on the heart was found in the literature surveyed.

Conclusive evidence of a chronic effect of sulfonamide therapy has not been obtained experimentally, but focal calcification and minor degrees of early fibroblastic proliferation have been noted in association with cellular infiltrates. In one case of periarteritis nodosa, secondary cellular infiltration attributable to sulfonamide drugs appeared comparable to that described in Rich's<sup>4</sup> report. Dense cellular infiltrations, hemorrhages, and focal necrosis might be expected to result in at least minor degrees of fibrosis. The focal myocardial calcification noted in 2 cases in the series may be regarded as a more permanent effect of the drug than the cellular infiltration and may be an example in human material of the lesions noted in rats fed sulfonamide by Endicott, Kornberg, and Daft.<sup>5</sup>

Hepatic infiltration and necrosis, with or without icterus, were frequent findings. When combined with lesions of the heart, kidneys, skin, and blood-forming organs, the fatal termination can be attributed to the severity of the total reaction.

The extensive pulmonary hemorrhages in 2 cases were comparable to hemorrhagic lesions encountered in the heart and kidney. The possibility of a relationship between hemorrhage by diapedesis and sensitivity to sulfonamide drugs must await further experimental confirmation. However, Pinkerton<sup>13</sup> and Gessler<sup>14</sup> have reported similar cases related to sulfonamide therapy.

Skin sections were taken in 4 instances of frank dermatitis. These 3 cases of erythema multiforme with vesiculation and one of erythema nodosum were striking. Loveman and Simon<sup>15</sup> reported a similar example of erythema nodosum in which the lesion was reproduced by repeated administration of the sulfonamide drug. It was significant that in 3 of the 18 cases that showed a skin rash dermatitis of severe proportions developed.

Skin reactions were the most striking clinical evidence of sensitivity to the sulfonamide drugs. From this series of cases the conclusion appears to be justified that any skin reaction other than simple erythema should be a contraindication to the continued use of any member of this

group of drugs. The utmost care must be exercised and adequate clinical study maintained if a patient who has had any type of skin reaction is subjected to further use of sulfonamides. Repeated courses of sulfonamide treatment given to a patient once shown to be sensitive appeared to magnify the severity of the complications, as 14 patients who exhibited sensitivity had had two or more courses of the drug. A change in form of the drug appeared to reduce the hazard if sulfonamide therapy was reinstituted, but even then the reaction might be severe or fatal.

The prophylactic use of sulfonamide drugs against meningitis, gonorrhea, and other infectious diseases may well result in sensitizing patients to the drug, as noted by Lyons and Balberor,<sup>16</sup> Nelson,<sup>17</sup> Kalz and Steeves,<sup>18</sup> and Stiles.<sup>19</sup> While this possibility should not be considered a contraindication to the prophylactic use of sulfonamides, it must be recognized if fatal reactions are to be avoided. The protection afforded the majority is the paramount consideration, and should not be disregarded because death occasionally occurs in a sensitized individual. On the other hand, the dangers of the indiscriminate use of sulfonamide drugs for prophylaxis or in the therapy of minor infections cannot be overemphasized.

### CONCLUSIONS

1. Striking histopathologic changes were seen in the material from 76 autopsies and in 2 additional specimens of skin taken for biopsy from patients who apparently had been sensitized to sulfonamides, as reviewed at the Army Institute of Pathology.

2. Characteristic acidophilic histiocytes were present in focal and diffuse infiltrations in the heart, liver, kidney, lung, spleen, lymph nodes, bone marrow, skin, testis, intestine, gallbladder, prostate, urinary bladder, skeletal muscle, thyroid, aorta, and meninges.

3. Significant vascular lesions were characterized by fibrinoid necrosis, endothelial edema, and proliferation.

4. Interstitial pneumonitis and hemorrhage by diapedesis in the pulmonary alveoli were attributed to sulfonamide sensitivity.

5. Focal subendocardial, subepicardial, and subpelvic renal hemorrhages were associated with typical acidophilic histiocytic infiltrates.

6. Sulfonamide crystals combined with calcium deposition were demonstrated in and adjacent to the distal convoluted and collecting renal tubules.

7. Evidence of individual susceptibility to initial and repeated courses of the sulfonamide group of drugs has accumulated in the literature and is substantiated by this series of cases.

8. Sensitization of large groups of patients with prophylactic doses of sulfonamide drugs may result in an increase in the number of histopathologic lesions encountered at autopsy. Many of these lesions were significant causes of death.

9. Increased caution must be observed in the prophylactic and therapeutic use of the sulfonamide drugs for minor infections.

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## DESCRIPTION OF PLATES

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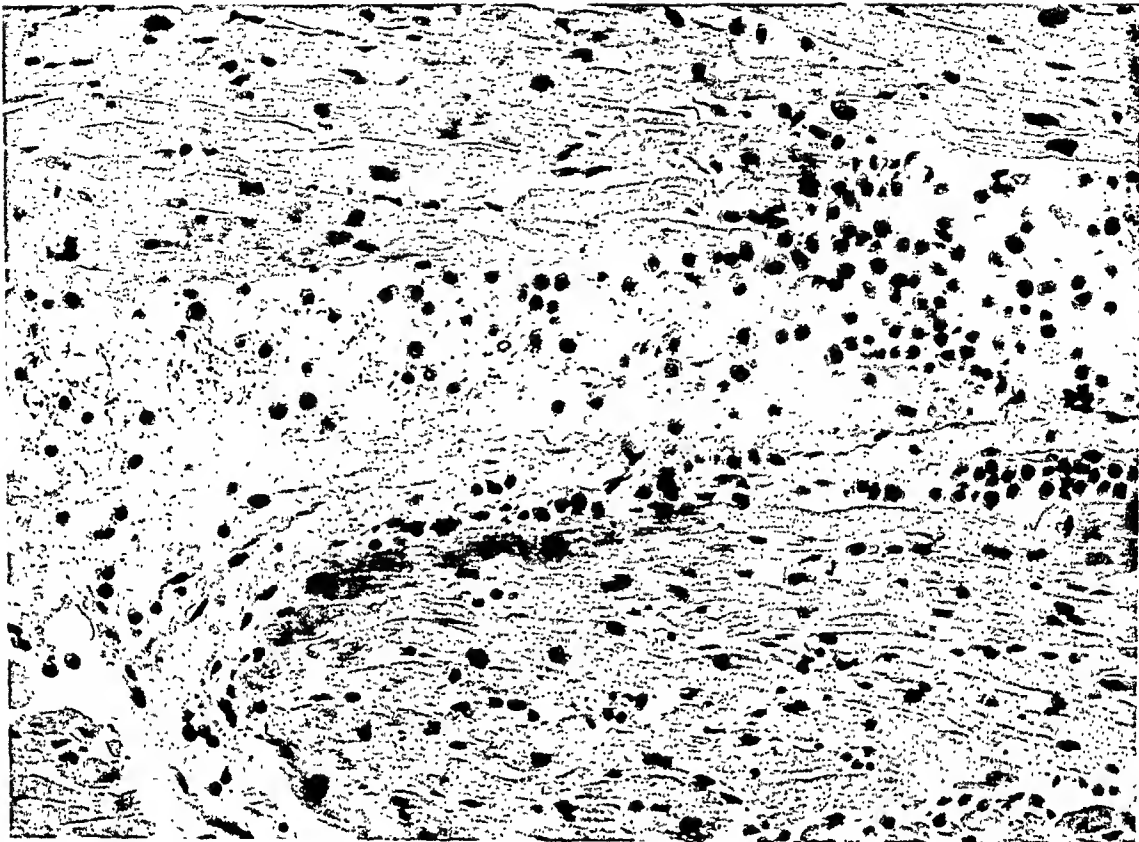
### PLATE 131

(A.I.P. = Army Institute of Pathology)

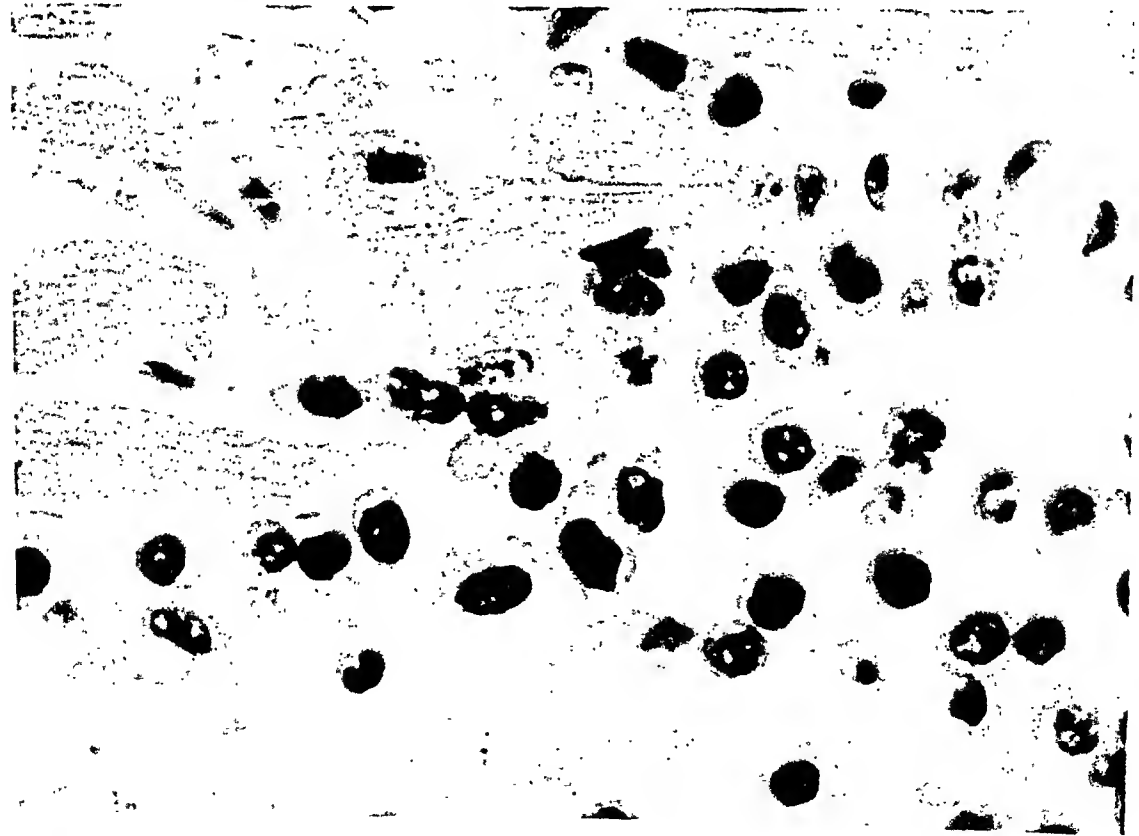
FIG. 1. Paravascular and interstitial infiltration of acidophilic histiocytes in the myocardium. Although monocytes predominate, a few polymorphonuclear cells are present also.  $\times 240$ . A.I.P. neg. 79036.

FIG. 2. A small area in the upper right quarter of the preceding illustration is shown at a higher magnification. The many mononuclear histiocytes which occupy the greater part of the field were chiefly acidophilic. A few cells of the granulocyte series are included in the infiltration. The muscle fibers are not necrotic.  $\times 1000$ . A.I.P. neg. 79039.

1



2



French

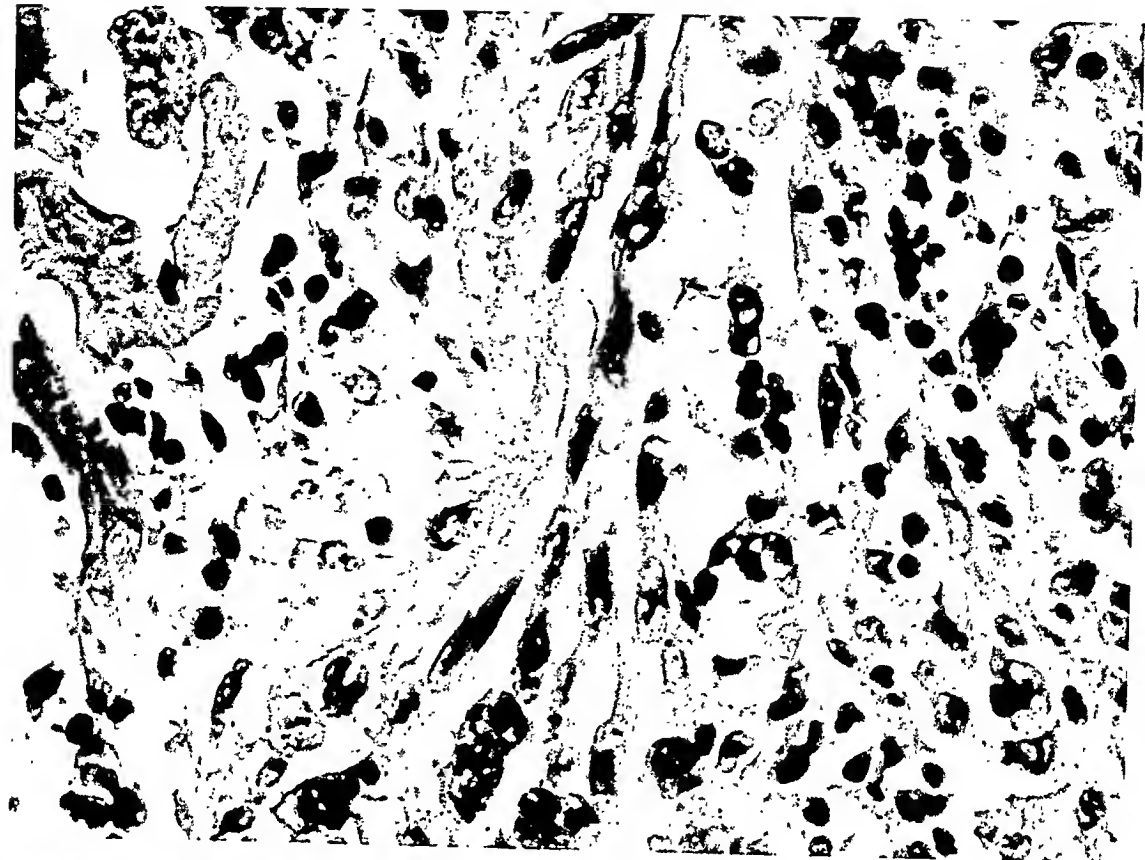
Histopathologic Changes with Sulfonamides



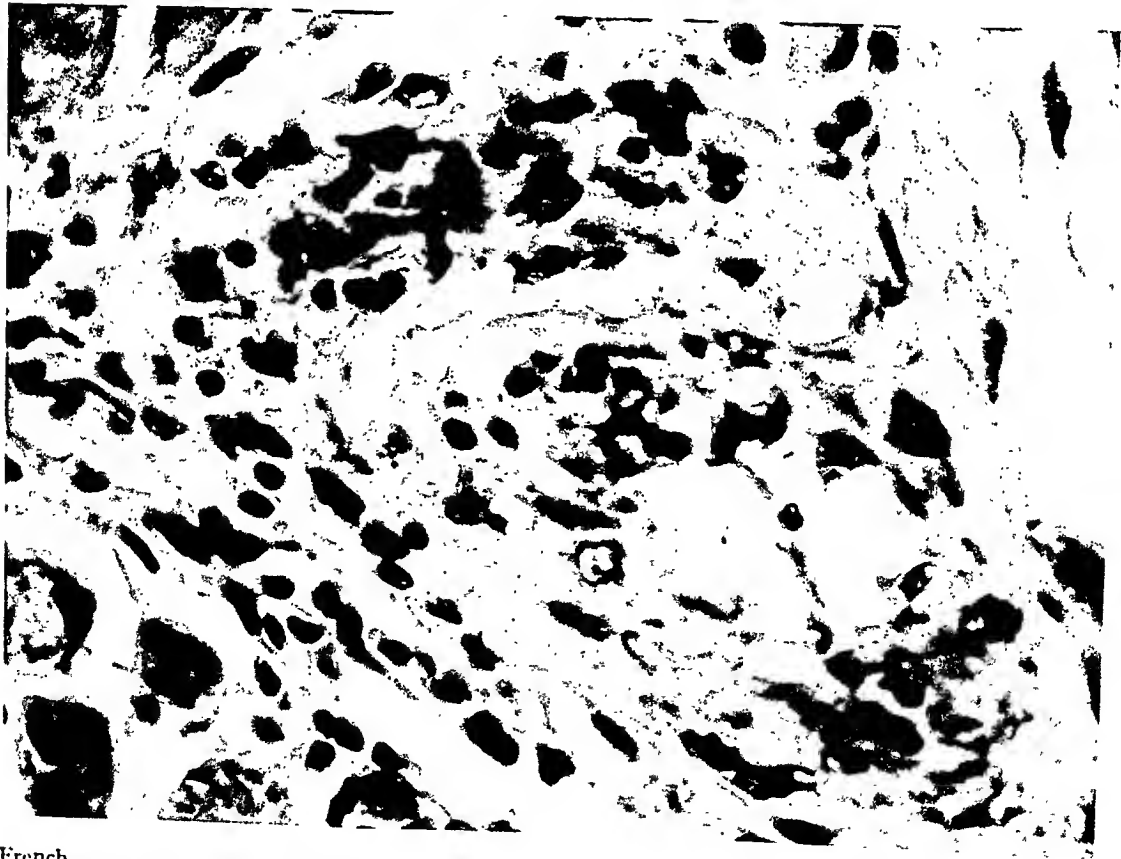
PLATE 132

FIGS. 3 and 4. Fibrinoid necrosis is present in the walls of capillaries in the myocardium. The paravascular tissues are edematous and infiltrated with acidophilic histiocytes. There is active proliferation of capillary endothelium.  $\times 700$ . A.I.P. negs. 79129 and 79132.

3



4



French

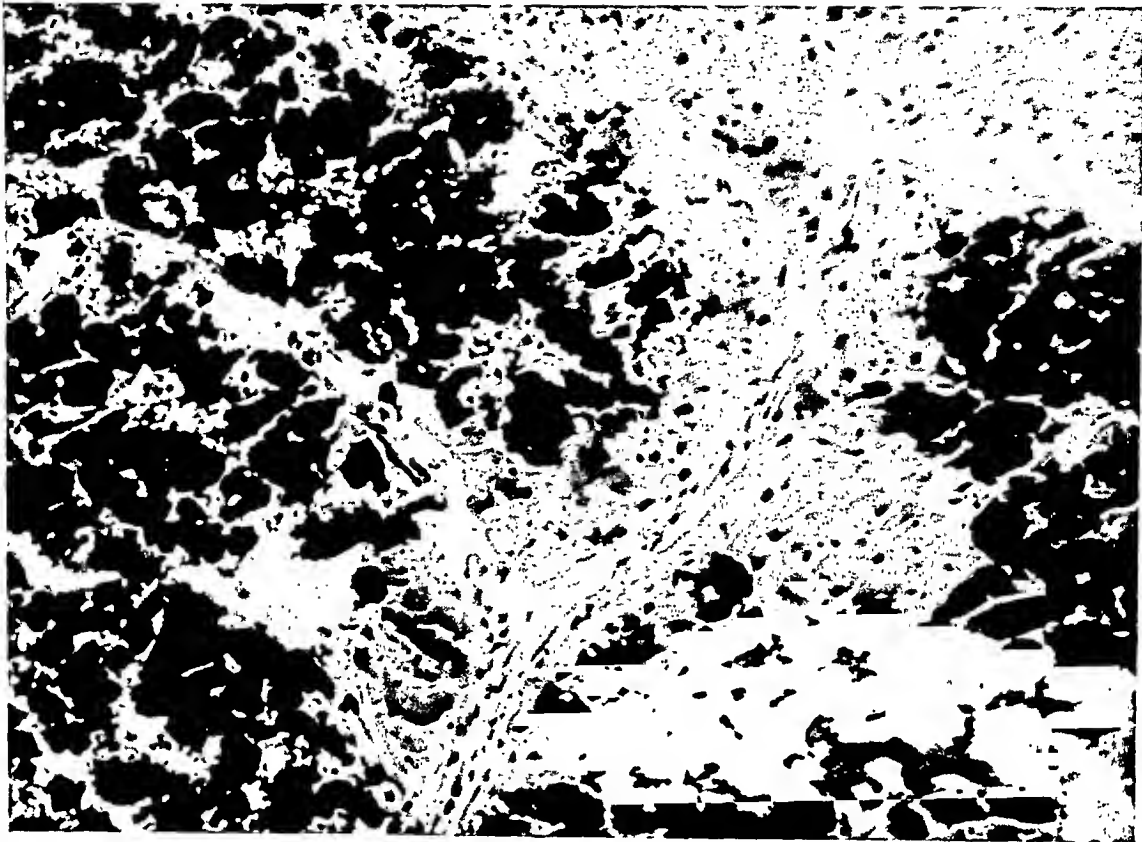
Histopathologic Changes with Sulfonamides

PLATE 133

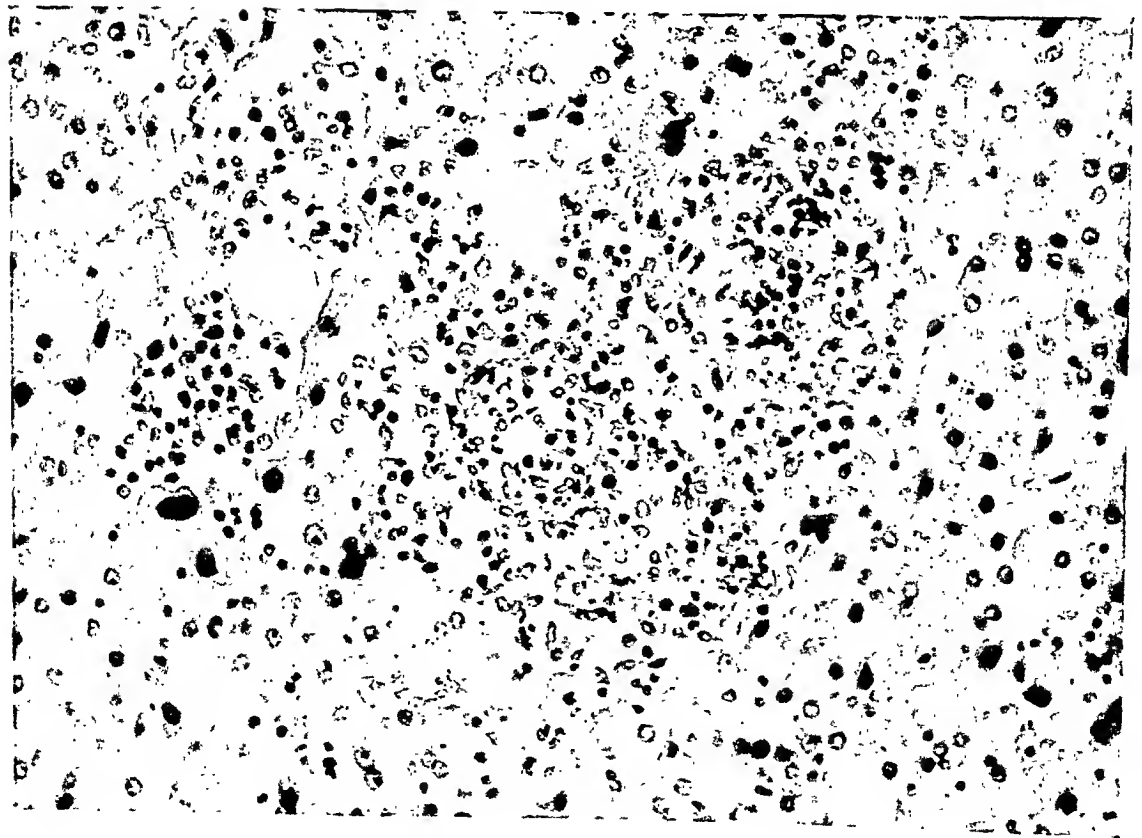
FIG. 5. Marked focal calcific deposits in myocardial fibers unassociated with primary vascular disease. The associated paravascular infiltrations of acidophilic histiocytes suggest a relationship to sulfonamide sensitivity.  $\times 130$ . A.I.P. neg. 79040.

FIG. 6. Focal necrosis of hepatic parenchyma with acidophilic histiocytes composing part of the exudate.  $\times 280$ . A.I.P. neg. 79041.

5



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French

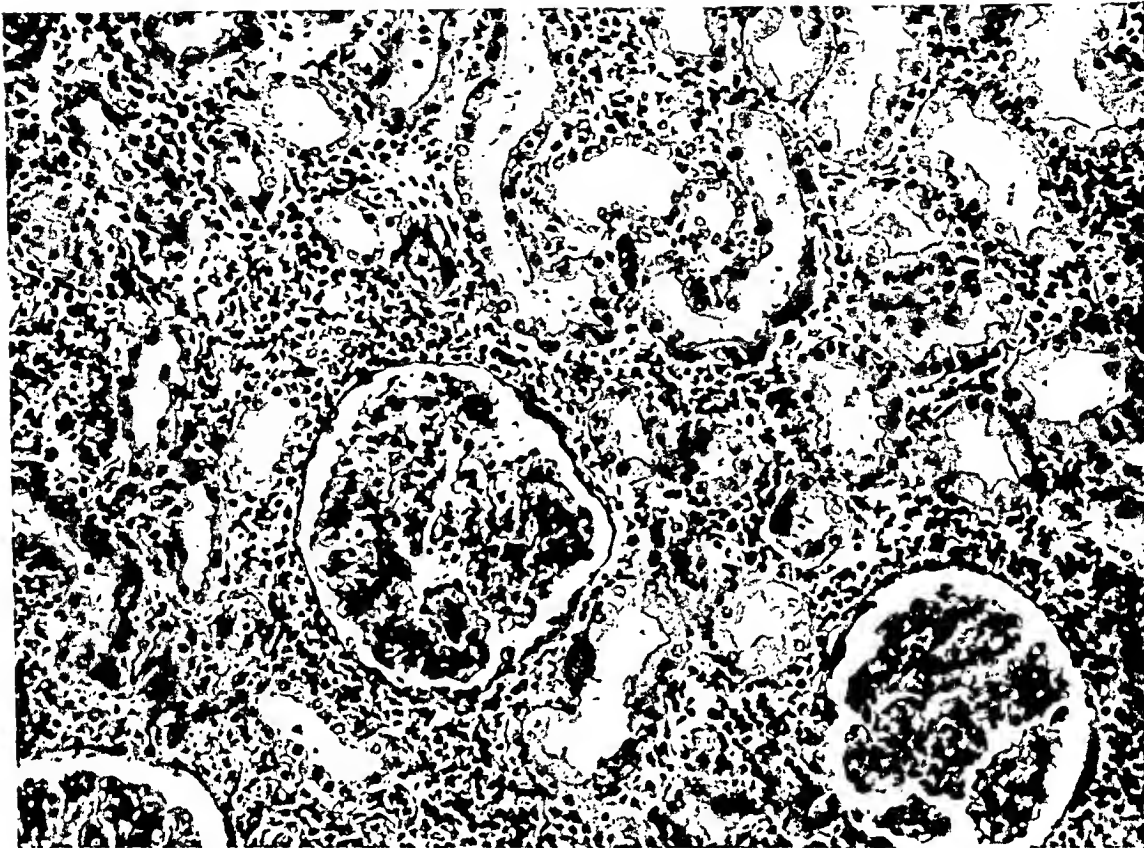
Histopathologic Changes with Sulfonamides

PLATE 134

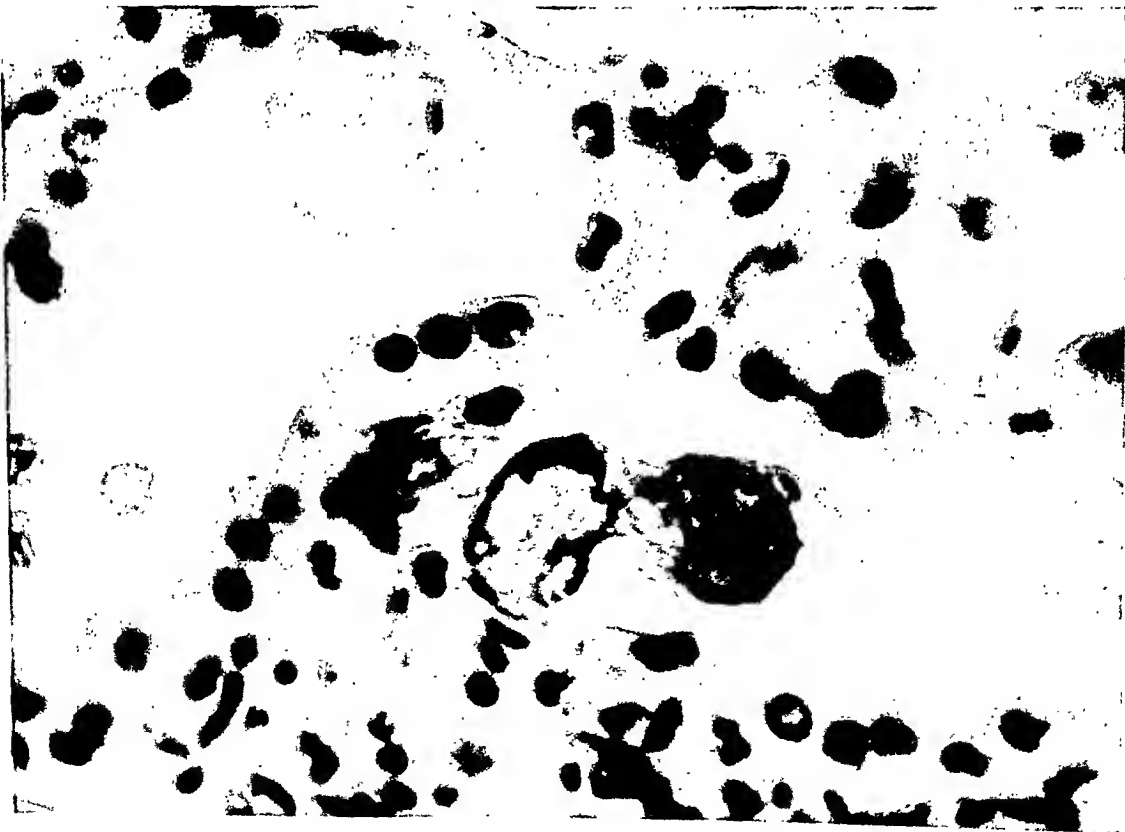
FIG. 7. Severe interstitial infiltration of acidophilic histiocytes and other inflammatory cells in the renal parenchyma. Acute parenchymatous degeneration of the epithelium of the tubules.  $\times 200$ . A.I.P. neg. 79131.

FIG. 8. Crystals of acetylated sulfathiazole with slight calcific deposition in renal tubules. Interstitial infiltration of acidophilic histiocytes.  $\times 915$ . A.I.P. neg. 79045.

7



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French

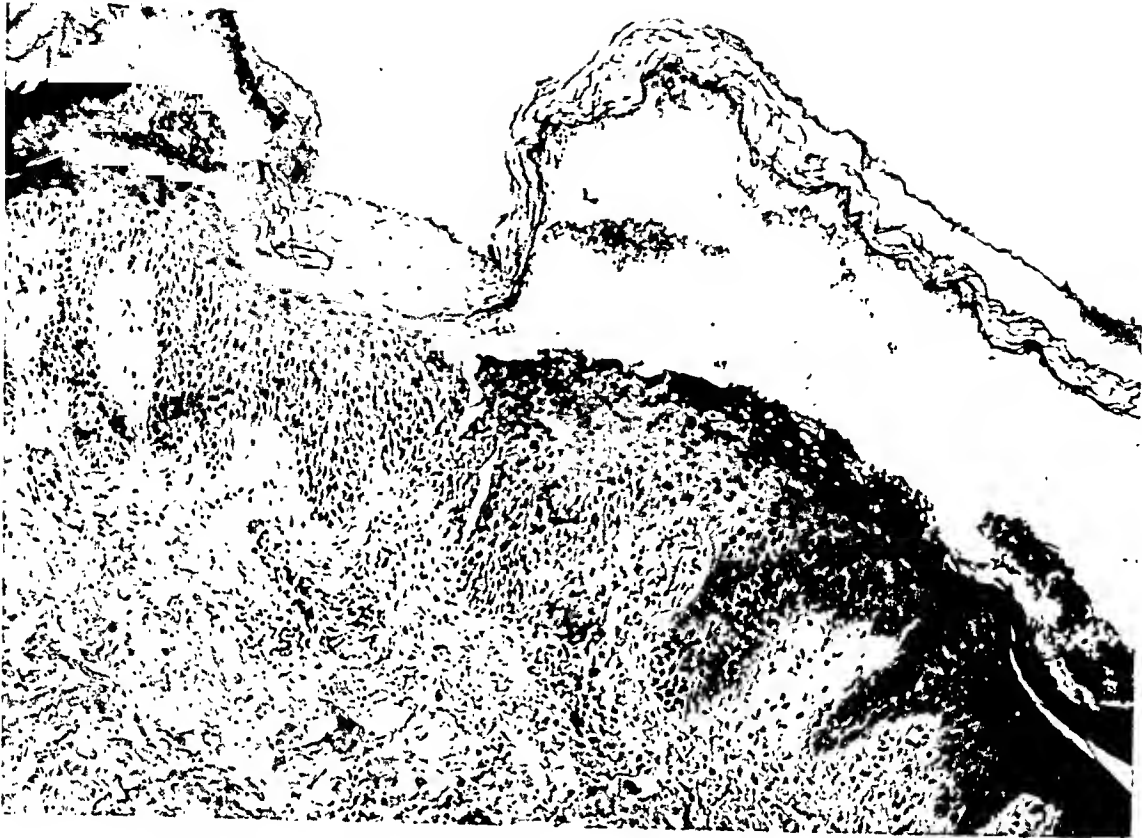
Histopathologic Changes with Sulfonamides

PLATE 135

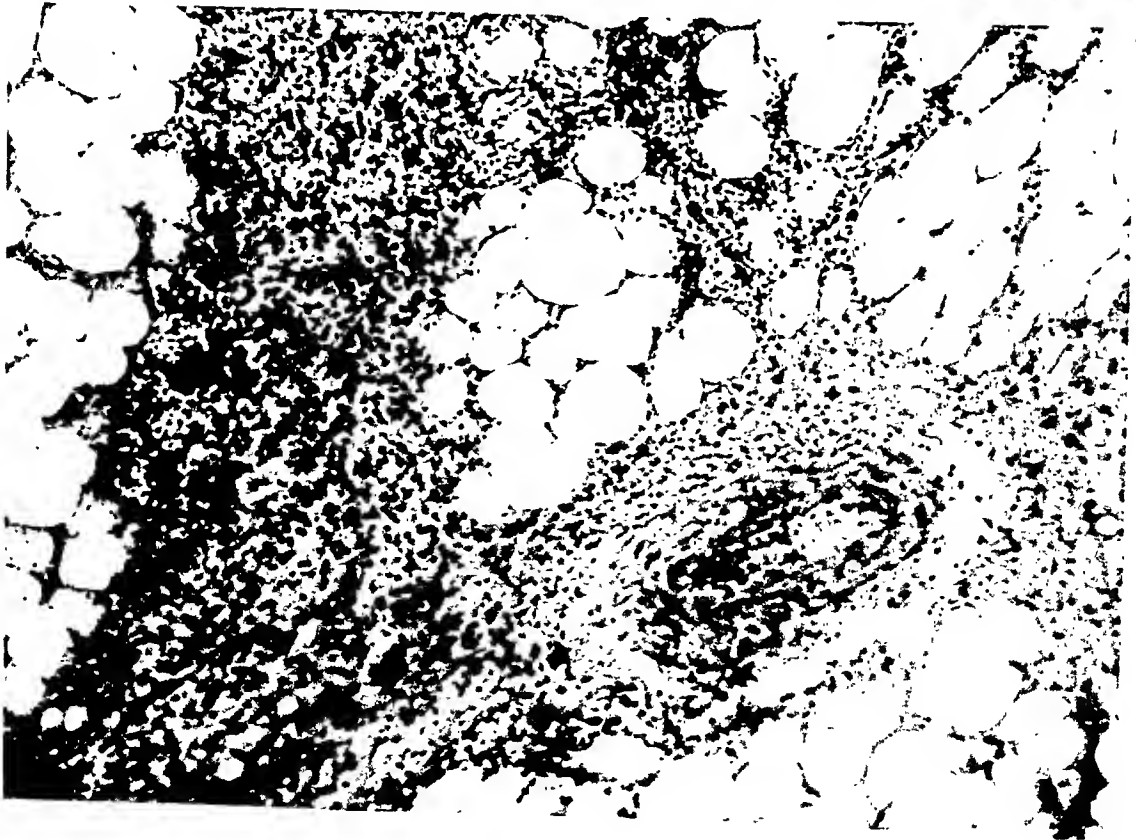
FIG. 9. Dermatitis with vesiculation in the superficial epidermis, resembling erythema multiforme and accompanied by a slight infiltration of acidophilic histiocytes in the dermis.  $\times 96$ . A.I.P. neg. 79044.

FIG. 10. Granulomatous focus in the subcutaneous adipose tissue of the type of erythema nodosum. The paravascular character of the infiltration is evident here as elsewhere.  $\times 120$ . A.I.P. neg. 79038.

9



10



French

Histopathologic Changes with Sulfonamides



PLATE 136

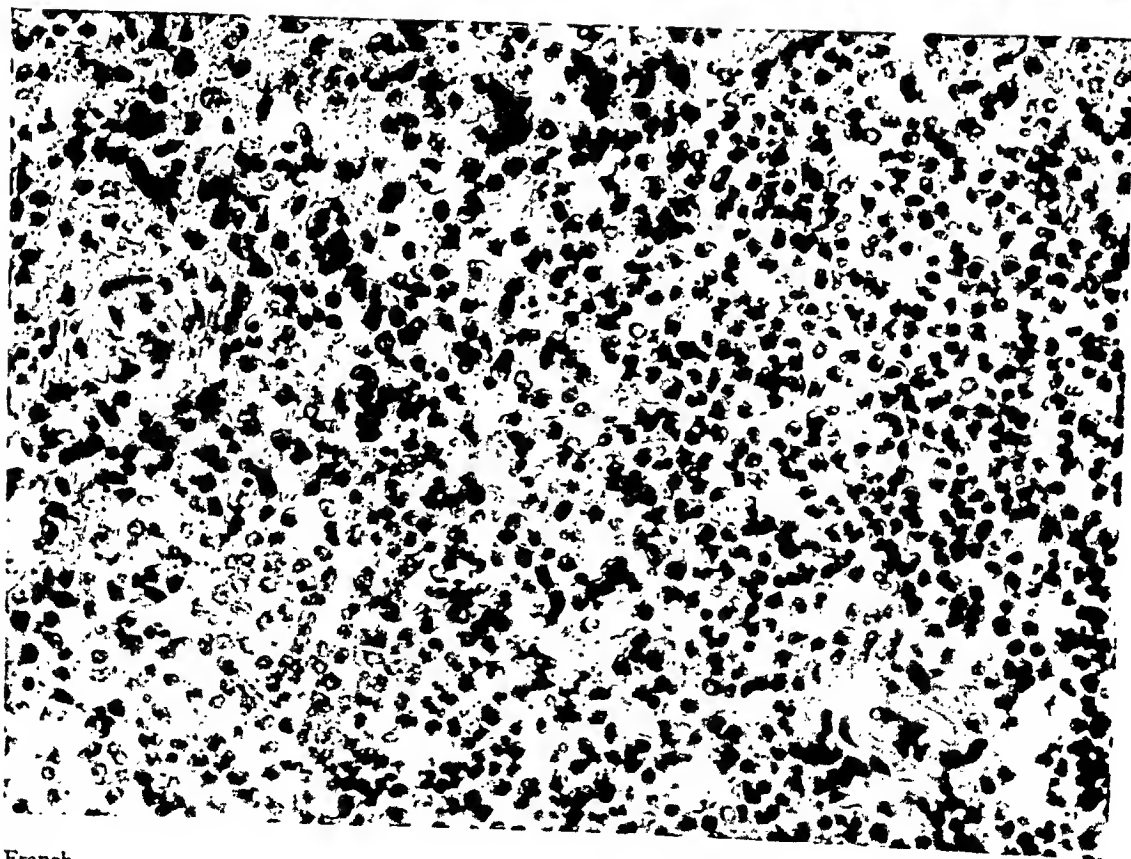
FIG. 11. Acidophilic histiocytes infiltrate the interstitial tissues of the testis and are readily distinguishable from the larger interstitial cells of Leydig. The adjacent germinal epithelium shows active maturation.  $\times 1000$ . A.I.P. neg. 79037.

FIG. 12. Small foci of necrosis are indicated in this area from the spleen by the chromatin dust which is present. Lymphocytes are decreased in the areas showing necrosis, and acidophilic histiocytes are abundant.  $\times 350$ . A.I.P. neg. 79046.

11



12



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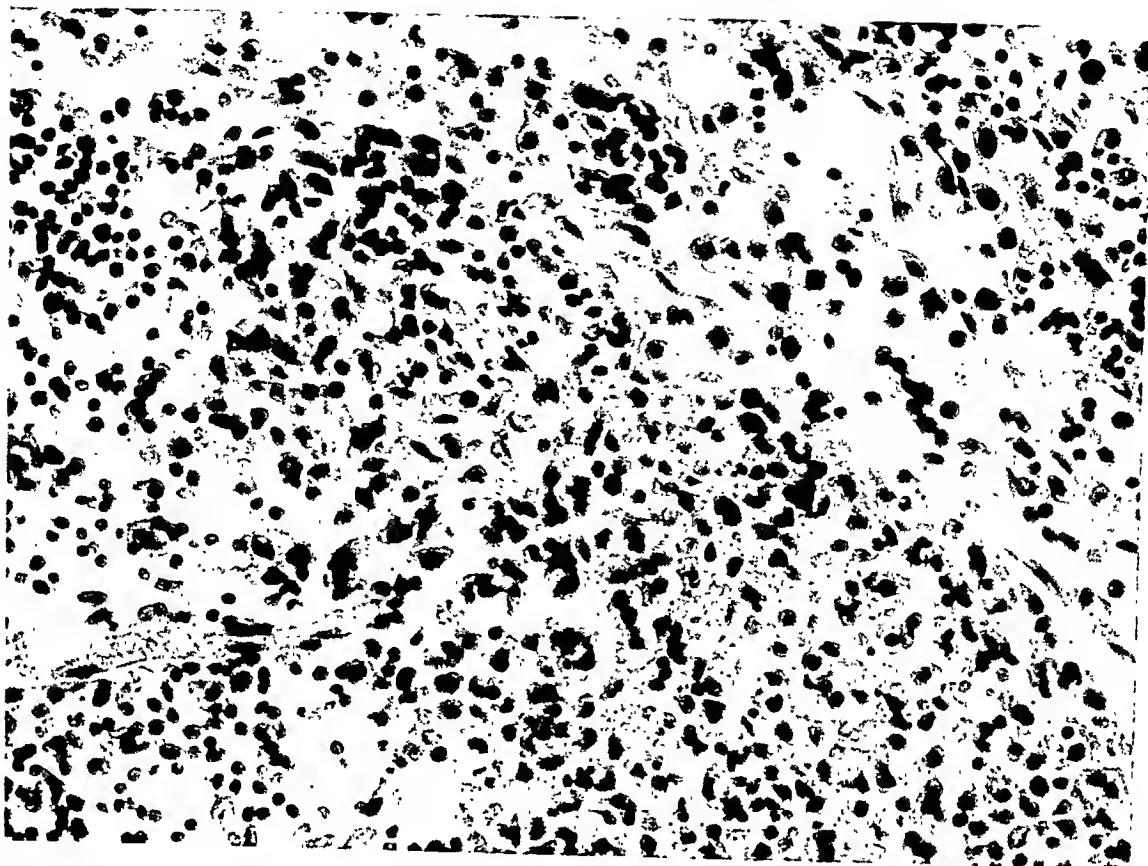
Histopathologic Changes with Sulfonamides

PLATE 137

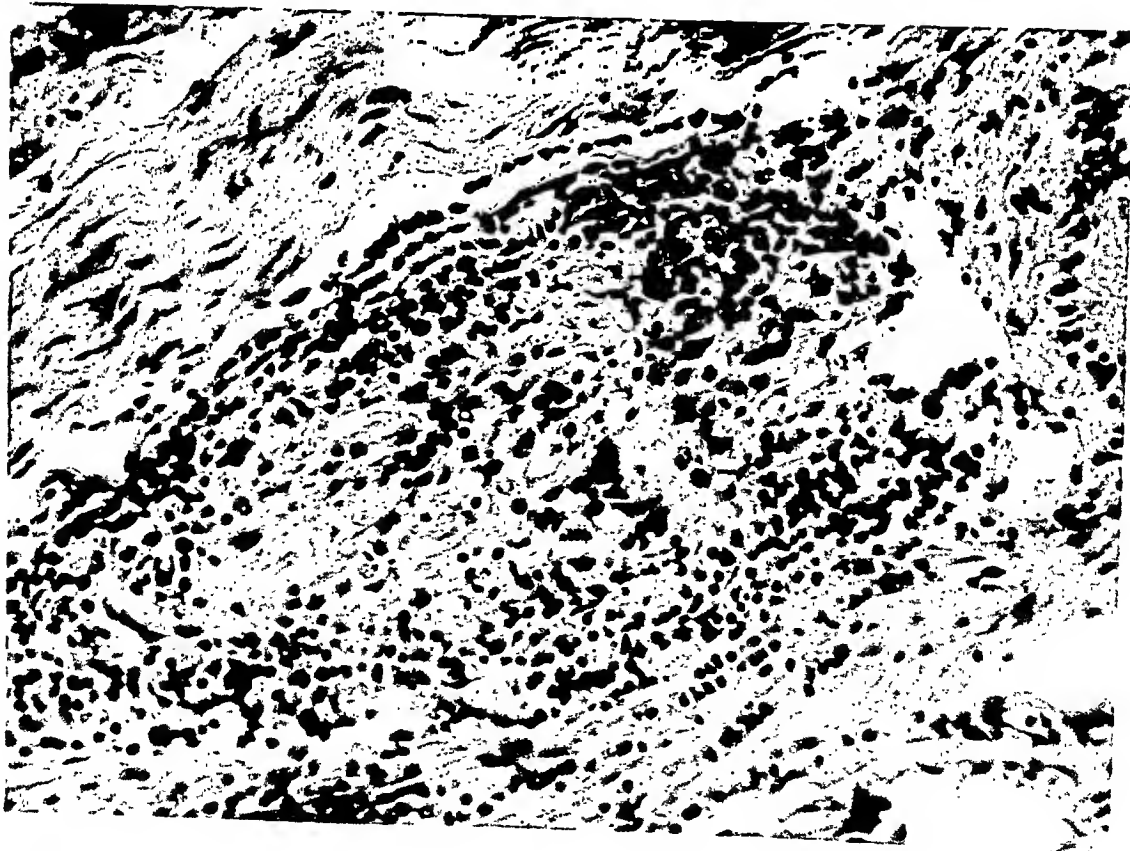
FIG. 13. Focal necrosis in a lymph node, with hyperplastic reticulo-endothelial cells. Acidophilic histiocytes are striking components of the cellular infiltration in the necrosed areas.  $\times 350$ . A.I.P. neg. 79043.

FIG. 14. A paravascular focus in the wall of the gallbladder which is granulomatous and resembles the lesions of periarteritis nodosa. The predominant cell is the acidophilic histiocyte.  $\times 330$ . A.I.P. neg. 79130.

13



14



French

Histopathologic Changes with Sulfonamides



# THE PATHOLOGY OF SULFONAMIDE ALLERGY IN MAN\*

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With the widespread use of the sulfonamide drugs, it soon became apparent that they sometimes caused untoward clinical results and even death.<sup>1,2</sup> A few of the patients who died had certain morphological changes that were attributed to sulfonamide therapy. Among the first of these lesions to be described were liver damage,<sup>3</sup> granulocytopenia,<sup>4</sup> and urolithiasis.<sup>5</sup> Further clinical and experimental studies rapidly enlarged the list of lesions caused by sulfonamide medication, and in 1942 French and Weller<sup>6</sup> showed that the heart and other organs could be affected. In 1943 Simon<sup>7</sup> presented a comprehensive review of the lesions attributable to the administration of the sulfonamide drugs. Since the publication of that review there have been many additions to the literature of sulfonamide lesions, but the emphasis, both clinically and pathologically, has remained upon the types of lesions reviewed by him. Those of the kidney, in particular, have continued to be reported frequently, while scant attention has been given to coexistent sulfonamide lesions.<sup>8</sup>

In the past few years 22 cases with sulfonamide lesions have been observed among the autopsies of this institute. The most common lesion encountered was a peculiar granulomatous reaction that has rarely been reported.<sup>9-11</sup> The series also contained several examples of a hitherto undescribed trabecular lesion of the spleen. Because this is the largest single group of cases of sulfonamide lesions yet studied, and because it contains many examples of both rare and common lesions, it provides a unique opportunity for the study of the morphology and pathogenesis of human sulfonamide lesions.

## MATERIAL AND METHODS

To obtain the present series of cases, 2,000 autopsy protocols for the years 1940 to 1944 inclusive were reviewed. The autopsy records showed that 375 of these patients had received sulfonamide drugs. It is obvious that many more patients among the 2,000 probably had received sulfonamides, but it was considered scarcely feasible to trace these. An examination of the records and pathological material of the 375 sulfonamide-treated patients yielded 22 cases in which lesions attributable to sulfonamides were present. It is to be emphasized that only outspoken lesions without other demonstrable cause were accepted.

\* Received for publication, December 4, 1945.

The 22 cases comprised 17 complete autopsies and 5 autopsies confined to the thorax and abdomen. Aerobic and anaerobic post-mortem blood cultures were done in 20 cases, while sections of sulfonamide lesions were stained to demonstrate acid-fast, Gram-positive, and Gram-negative organisms in all cases.

A control series of cases not treated with sulfonamide drugs was established by reviewing 400 autopsy cases from the pre-sulfonamide years of 1930 and 1931. These cases were consecutive and unselected save in two particulars only: partial autopsies and examinations of infants less than 1 month of age were excluded. Microscopical sections of the heart, liver, spleen, and kidneys were examined. Sections of bone marrow were not available. It can be stated at once that no lesions similar to those to be described in this paper were found in the control series except in association with an etiological agent that is known to produce such lesions, as, for example, the interstitial nephritis found in cases of scarlet fever, the myocarditis associated with diphtheria, and the granulomata of miliary tuberculosis.

#### GENERAL ANALYSIS OF CASES

An analysis of individual cases is presented in Table I. A more general consideration of the cases, as presented below, reveals that, preceding the administration of the sulfonamides, there were no factors common to these patients that appeared to determine the development of the lesions in question.

The patients were commonly of middle age, while the age extremes were 5 and 84 years. Eighty-two per cent were males in contrast to an autopsy population of 65 per cent males. The cases were drawn almost equally from the surgical and medical services, the clinical diagnoses being correspondingly diverse. At autopsy the state of nutrition was variable, 12 of the patients being well nourished, 6 being fairly well nourished, and 4 being in a state of poor nutrition.

While sulfathiazole was the sulfonamide compound most commonly employed, sulfanilamide, sulfapyridine, sulfadiazine, and sulfasuxidine were used in some cases. Daily dosages were always of the ordinary therapeutic amounts; total dosages varied from 75 to 3,100 grains.

Following the administration of these drugs several of the patients exhibited interesting and unexpected reactions which are detailed below. Four of the patients showed reactions that were obviously of an allergic nature. An additional 7 patients showed definite and frequently severe responses to the drugs, either during a long continuous course of therapy or, in some instances, immediately upon the institution of a further course of therapy. Two of the patients, cases 6 and 13, pre-

TABLE I  
Analysis of Cases Showing Sulfonamide Lesions

Case no.	Clinical data					Sulfonamide lesions				
	Age and sex	Reason for sulfonamide therapy	Total dose (grains)	Cause of death (autopsy)	Remarks	Heart	Liver	Spleen	Kidneys	Other organs
1	33 M	Meningitis	1230 C* sulfapyridine	Granulocytopenia	Terminal febrile response with angina and cystitis; white blood cells, 1,600; red blood cells, 2,000,000; no granulocytes					Aplasia and focal necrosis of bone marrow
2	54 M	Supposed septicemia	195 C sulfapyridine	Myocardial failure and complications	No clinical reactions	Interstitial myocarditis; granulomata				
3	40 M	Gonorrhea	1500 C sulfapyridine	Granulocytopenia	Angina, jaundice, fever and delirium; white blood cell count rising after withdrawal of drug					Immaturity of bone marrow
4	31 M	Sinusitis	3 weeks' course of sulfanilamide	Acute glomerular and interstitial nephritis	Skin rash, conjunctivitis, swollen joints and fever; no drug for 7 weeks before death			Polyvasculitis; trabeculitis	Interstitial nephritis	
5	58 M	Postoperative fever	180 C sulfapyridine	Postoperative death (acoustic neuroma)	Hemorrhagic and exfoliative dermatitis		Granulomata	Trabeculitis		Dermatitis
6	84 M	Perineal inflammation	390 C sulfanilamide, sulfapyridine, sulfathiazole	Perineal infection, terminal pneumonia	Jaundiced for 5 days before death				Interstitial nephritis; polyvasculitis	Hyperplasia and immaturity of bone marrow



TABLE I—(Continued)  
Analysis of Cases Showing Sulfonamide Lesions

Case no.	Clinical data					Sulfonamide lesions				
	Age and sex	Reason for sulfonamide therapy	Total dose (grains)	Cause of death (autopsy)	Remarks	Heart	Liver	Spleen	Kidneys	Other organs
7	37 M	Multiple traumata	585 C sulfathiazole	Traumata	No clinical reactions	Interstitial myocarditis; granulomata				Focal necrosis of bone marrow
8	66 M	Fever (simple fracture of leg)	1170 I <sup>†</sup> sulfathiazole	Polyvasculitis	Symptoms of vasculitis and vomiting with second course; terminal convulsions	Interstitial myocarditis	Granulomata; polyvasculitis	Trabeculitis	Interstitial nephritis; polyvasculitis; glomerulitis	Granuloma of fracture callus
9	48 M	Fever	338 C sulfathiazole	Exsanguination from acute peptic ulcer	No clinical reactions		Focal necrosis and hepatitis			
10	19 F	Pansinusitis and pneumonia	1365 I sulfathiazole, sulfadiazine	"Toxemia" and polyvasculitis	No clinical reactions			Trabeculitis	Interstitial nephritis; glomerulitis; granulomata	Polyvasculitis of sinuses and lungs
11	68 M	Fever	310 I sulfanilamide, sulfathiazole	Localized peritonitis, intestinal obstruction, sulfonamide lesions	No clinical reactions	Interstitial myocarditis; granulomata	Focal necrosis; granulomata		Granulomata	Focal necrosis of bone marrow; granulomata of peritoneum; polyvasculitis of prostate
12	50 M	Empyema thoracis	1170 C sulfathiazole	Perforated peptic ulcer, sulfonamide lesions	No clinical reactions	Granulomata		Trabeculitis	Interstitial nephritis; granulomata	
13	56 M	Prophylactic (carcinoma of urinary bladder)	75 I sulfathiazole	Massive pulmonary embolism	Questionable febrile reactions		Granulomata		Granulomata	

Clinical data						Sulfonamide lesions				
Case no.	Age and sex	Reason for sulfonamide therapy	Total dose (grains)	Cause of death (autopsy)	Remarks	Heart	Liver	Spleen	Kidneys	Other organs
14	68 M	Septic course	345 I sulfathiazole	Renal failure, myocarditis	No clinical reactions	Interstitial myocarditis	Granulomata		Granulomata	
15	42 F	Pneumonia	418 I sulfathiazole	Heart failure, polyvasculitis	Temperature rose 3.8 F. 24 hours after first dose of second course		Polyvasculitis; granulomata	Trabeculitis	Granulomata	Granulomata of lung and bronchus
16	5 M	Upper respiratory infection	165 C sulfadiazine	Postoperative (C.N.S.) death	No clinical reactions		Granulomata			
17	31 M	Meningitis	450 C sulfadiazine	Tuberculous meningitis	No clinical reactions					Polyvasculitis of renal pelvis
18	47 F	Prophylactic	370 C sulfathiazole	Massive pulmonary embolism	Asthmatic attacks; history of asthma				Interstitial nephritis; granulomata	
19	63 F	Empyema thoracis	1260 I sulfathiazole, sulfadiazine	Massive hepatic necrosis	Jaundiced 2½ days after first dose of final course; immediate fall of fever; died in 6 days		Massive necrosis			
20	58 M	Prophylactic	232 I sulfathiazole	Massive hepatic necrosis	Jaundiced 28 hours after first dose of second course; history of bronchitis; died in 3 days		Massive necrosis			
21	51 M	Ulcerative colitis	3100 I sulfasuxidine	Colitis and sulfonamide lesions	Conjunctivitis; sulfonamides 1 year previously	Interstitial myocarditis; granulomata	Granulomata		Interstitial nephritis; granulomata	Focal necrosis of bone marrow
22	49 M	Postoperative pneumonia	150 C sulfathiazole	Sulfonamide reaction with anuria	Chill, fever, oliguria, delirium, convulsions; sulfonamides 2 years previously				Nephrosis	

\* C—Continuous course of therapy.

† I—Interrupted course of therapy.

sented reactions of a suggestive but questionable character. The remaining 9 patients suffered no unfavorable clinical reaction to the sulfonamides.

The 4 patients who exhibited clinical allergic reactions were as follows:

*Case 4.* This patient was given a 3 weeks' course of sulfanilamide for sinusitis. At the termination of the course there developed painful and swollen joints, a skin rash, conjunctivitis, and fever. He lived for 7 weeks, receiving no further sulfonamide.

*Case 5.* This patient was given 180 grains of sulfapyridine because of a fever that occurred after the removal of an acoustic neuroma. A severe hemorrhagic and exfoliative dermatitis developed which dermatological consultants attributed to the sulfonamide.

*Case 18.* This patient had acute cholecystitis and was given 370 grains of sulfathiazole in a continuous course. There was a history of asthma, and the drug appeared to induce asthmatic attacks.

*Case 21.* This patient suffered from chronic ulcerative colitis. On two occasions during the previous year he had been given sulfonamides without untoward reactions. On his final admission he received 3,100 grains of sulfasuxidine in an interrupted course. Four days before death conjunctivitis developed.

The remaining 7 patients who suffered untoward clinical reactions to sulfonamide therapy are as follows:

*Case 1.* This patient presented signs and symptoms leading to a diagnosis of meningitis. He was given 1,230 grains of sulfapyridine in a continuous course. Terminally, angina and cystitis developed, while his white blood cell count fell from a high level to 1,600 and no polymorphonuclear leukocytes could be found in the blood. The red blood cell count fell to 2,000,000. The temperature rose from 98° to 107°F. during the terminal 6 days (Text-Fig. 1-a).

*Case 3.* This patient was given 1,500 grains of sulfapyridine in a continuous course of treatment for gonorrhea. When admitted to the hospital there were granulocytopenia, jaundice, angina, fever, and delirium.

*Case 8.* This patient was hospitalized for a simple fracture of the tibia and fibula. He was given 1,170 grains of sulfathiazole in an interrupted course for an upper respiratory infection. His initial exposures to the drug were without untoward reactions, but the final course caused nausea and vomiting, while the signs and symptoms of periarteritis nodosa developed at the same time.

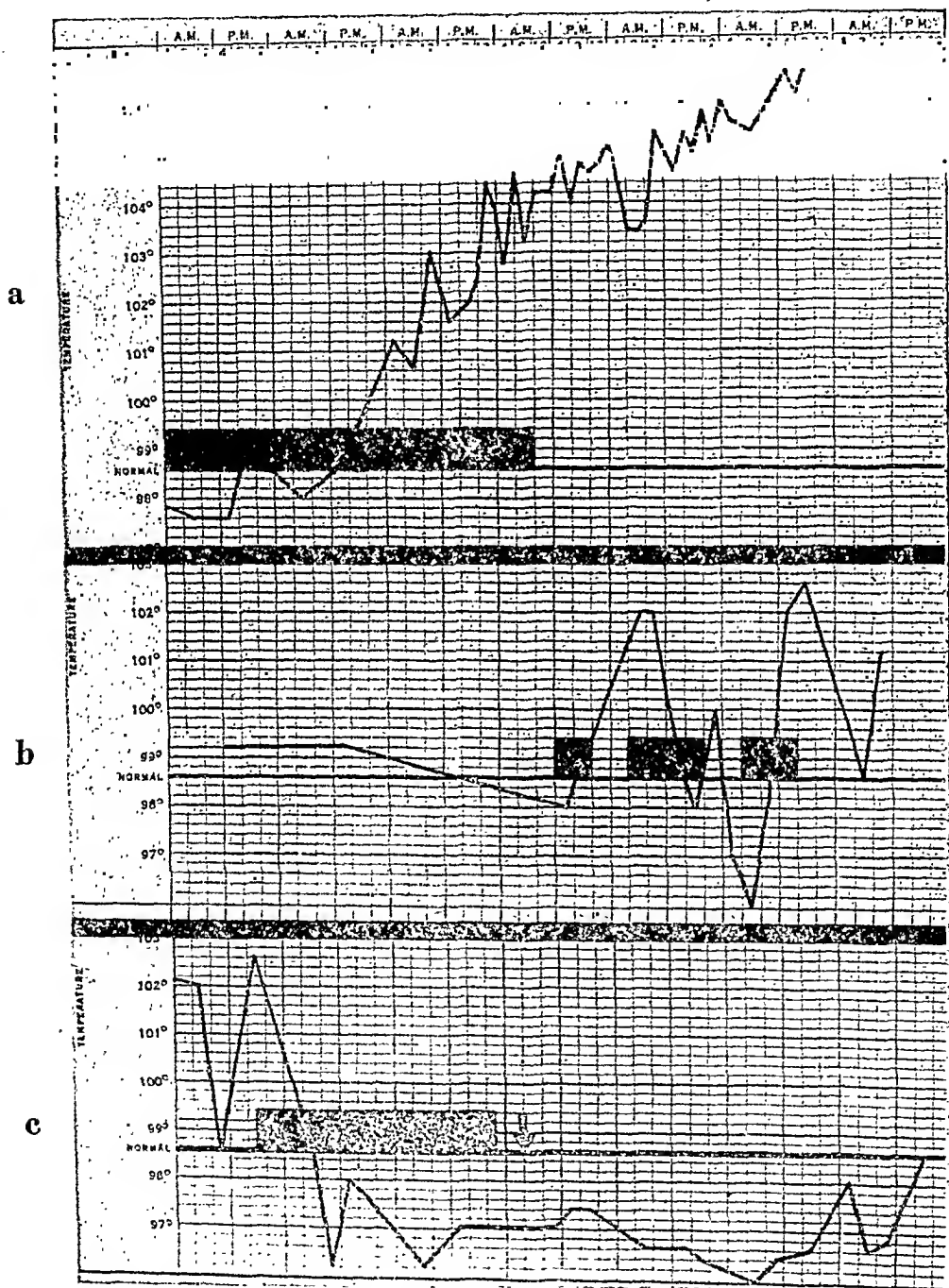
*Case 15.* This patient had coronary heart disease and was given 418 grains of sulfathiazole in an interrupted course for suspected pneumonia. The initial exposure to the drug caused no untoward reaction, but the final course, administered after an interval of 3½ weeks, caused the temperature to rise 3.8°F. within 24 hours (Text-Fig. 1-b).

*Case 19.* This patient had an empyema thoracis and was given 1,260 grains of sulfathiazole and sulfadiazine in an interrupted course over a period of 2 months. The first courses of therapy were without unusual reactions, but jaundice occurred 2½ days after the first dose of the final course was given, and the patient died 3½ days later from acute hepatic insufficiency (Text-Fig. 1-c).

*Case 20.* This patient had a very similar reaction to that in case 19. He was given 232 grains of sulfathiazole in an interrupted course. Again, there were no untoward reactions with the first exposure, but jaundice appeared 28 hours after the first dose of the second course and the patient died of acute hepatic insufficiency within 3 days. This patient had a history of seasonal bronchitis.

*Case 22.* This patient was known to have received sulfonamides 2 years pre-

viously with satisfactory effect. On his final admission he was operated upon for the repair of a ventral hernia. On the day following operation he produced 470 cc. of urine and was given sodium sulfathiazole in glucose-saline solution intravenously. One-half hour after this treatment the patient suffered a chill; delirium, convulsions, and severe oliguria followed. On the 3 succeeding days he produced 195, 22,



Text-Fig. 1 (a, b, and c). Terminal temperature readings of cases 1, 15, and 19. The heavy black blocks indicate the period of sulfonamide therapy. The arrow in Figure 1-c indicates the development of visible jaundice.

and 50 cc. of urine. Death occurred 4 days after the sulfonamide was first given. A total of 150 grains of the drug was used. Clinically, death was attributed to a severe sulfonamide reaction.

Among the 22 cases in which sulfonamide lesions were found, it is our opinion that these lesions caused the death of 7 patients, were a major factor in the death of 7 patients, and were of minor or negligible importance in the death of 8 patients.

### PATHOLOGY OF SULFONAMIDE LESIONS

The lesions encountered in the 22 cases, and which we ascribe to sulfonamide therapy, fall in the main into the morphological classification of necrotic, granulomatous, interstitial inflammatory, and polyvascular inflammatory groups, and a small miscellaneous group. In many cases the lesions were not purely of one type, and combined or intermediate forms existed. In most cases the lesions were microscopic. In only 3 instances were they visible to the naked eye. The gross appearance of the sulfonamide lesions in these 3 cases will be described under their appropriate headings below.

#### *Necrotic Lesions*

Necrosis of the liver was the predominant lesion in 3 cases, while focal necrosis of the bone marrow was observed in 3 other instances. In all types of sulfonamide lesions studied, severe degeneration and necrosis were always minor and variable concomitants.

*Liver.* Two cases of massive hepatic necrosis presented in the gross a shrunken, soft liver with a wrinkled capsule of mottled yellow and purple color. On section one liver presented a fairly uniform, smudgy, yellow surface with loss of the normal markings. The cut surface of the liver in the other case showed patchy yellow areas with a loss of normal markings. These areas were irregular in outline, measured up to 4 cm. in diameter, and were often confluent. Microscopically, this extensive necrosis took the form of widespread, confluent central necrosis associated with hemorrhage into the areas of necrosis, and marked hyperemia of the sinusoids throughout the rest of the liver. The spaces of Disse were ballooned with amorphous eosinophilic material. In the areas of recent necrosis an almost normal outline of liver cells was retained, but their cytoplasm was waxy and eosinophilic and the nuclei had either disappeared or showed karyolysis. The most striking feature of these necrotic areas was the good preservation of the Kupffer cells and the increased number of nucleated cells in the sinusoids of the necrotic areas and of the adjacent better-preserved areas. Some of the cells were polymorphonuclear leukocytes, but for the most part they were large mononuclear cells, representing either an accumu-

lation of blood-borne monocytes, or a proliferation of reticulo-endothelial cells *in situ*. In the greater portion of both livers the necrosis was so extensive and advanced that the liver simulated closely the gross and microscopical appearance of acute yellow atrophy. However, there was no evidence of regeneration of liver cells or bile ducts. Only a few liver cells about the portal areas and a few of the perilobular bile ducts maintained a normal appearance. Reticulin stains revealed a remarkably good preservation of the architecture of the reticulin framework, and this probably accounted for the relatively good preservation of the liver cord architecture. The liver in the third case, while showing necrosis, might be classified equally well as an example of interstitial hepatitis. The lesion in this instance showed a very severe focal degeneration of liver cells. Individual liver cells displayed eosinophilic necrosis or dissolution associated with marked disorganization of the liver cords. The most striking feature of this lesion was the large number of well preserved mononuclear cells in the sinusoids between the necrotic liver cords. The sinusoids were filled with eosinophilic debris containing a few polymorphonuclear leukocytes and eosinophils. There was a very close similarity between this lesion and those seen in the less damaged areas of the livers of the 2 cases described above, and it appeared to be merely a phase in the development of the more massive necrosis (Fig. 1).

*Bone Marrow.* Focal necrosis of bone marrow was associated in one case with aplasia and in 2 with a very cellular marrow, exhibiting immaturity of the myeloid elements. These areas of necrosis appeared as irregular but well circumscribed pale patches. This pallor was due to a loss of cells and of the normal reticular structure which was replaced by an amorphous mass of eosinophilic tissue debris. There were no pyknotic nuclei in the necrotic areas of the hypoplastic marrow. However, in these necrotic areas there were even fewer cells than in the rest of the marrow. In the two cellular bone marrows showing immaturity, the pale patches of focal necrosis were pronounced, and in these the washed out background was peppered with many irregularly fragmented and pyknotic nuclei. The areas of focal necrosis in all cases failed to show the normal reticular framework with special stains. Moreover, in the hypoplastic marrow, the widespread fuzzy, granular, disrupted appearance of the supporting framework was associated with a loss of the reticulin network as demonstrated by special silver stains.

### *Granulomatous Lesions*

Lesions of granulomatous character were those most commonly encountered in our series, occurred in a well advanced stage in 13 of the 22 cases, and involved a total of 25 organs. They were found in the

heart in 5 cases, in the liver in 8, in the kidneys in 8, and in the lung and bronchus, the callus of a fracture, and in the peritoneum, each in one case. In only one case (no. 21) were these lesions visible grossly. In this case they could be seen in the heart, liver, and kidneys, and in these organs they formed well demarcated gray spots varying in size from a barely visible speck up to 3 mm. in diameter. The granulomata were, in many cases, associated with minimal to advanced degeneration of the parenchyma of the organ involved. In some cases an interstitial inflammation accompanied the granulomatous reaction. There was no difficulty in separating the two entities on morphological grounds although a pathogenetic distinction was not so apparent.

The granulomata, in whatever situation they occurred, were definitely of focal character with an irregular but well defined outline. The architecture of the adjacent parenchyma was usually well preserved, even though some degree of cytoplasmic degeneration existed in the nearby cells (Figs. 2 to 4). Characteristically, the granulomata presented a uniform cellular pattern from the center to the periphery of the lesion, producing a tessellated appearance. This mosaic appearance was due to the uniform and closely packed arrangement of large mononuclear cells with somewhat irregular, blunt, basophilic nuclei set closely and uniformly. The cytoplasm of these cells was abundant but pale (Fig. 4). The dominant cell of the lesion was always of a large mononuclear type. In addition, there were lesser and varying numbers of plasma cells, lymphocytes, polymorphonuclear leukocytes, and eosinophils.

The eosinophilic background of the lesion consisted of the necrotic parenchyma of the organ involved. This necrosis was usually so complete that it showed a total loss of the original cells and architecture, and there remained only a granular, eosinophilic mass of debris. In this background pyknotic and fragmented nuclei were seen, presumably derived also from the necrosis of the original cells of the parenchyma. Eosinophils, when present, were easily identified and were sometimes very numerous. However, since they were often absent, they were not considered a necessary characteristic of the lesion. In the earliest stage of development of the granulomata, partially preserved remnants of the original parenchyma were still present (Fig. 2). Sometimes within one organ well developed granulomata were associated with other focal areas of varying degrees of degeneration and cellular infiltration, which we regarded as representing various stages in the development of the granuloma. Some of these focal areas presented only focal degeneration, while others showed many necrotic cells with a slight mononuclear cellular infiltration, and, finally, there were some

areas consisting predominantly of large mononuclear cells among which an occasional necrotic cell of the original tissue could be seen (Fig. 3).

*Heart.* In the heart the granulomatous lesions were found occasionally in the intermuscular septa extending between adjacent muscle fibers. More often they replaced areas of muscle in the myocardium. In the center of these latter lesions the myocardial fibers were completely lost although reticulin stains revealed that the sarcolemmal sheaths were preserved in the midst of the cellular reaction. At the margin of these focal lesions the muscle fibers were usually necrotic, presenting the appearance of Zenker's necrosis (Figs. 5 and 6). These necrotic fibers were separated by a light infiltration of varying inflammatory cells (Fig. 5), producing a somewhat similar appearance to the interstitial inflammation of the heart to be described later.

*Liver.* In the liver the granulomata were most often found in the portal areas, but they also occurred with less frequency in all other parts of the lobule. In the involved portal areas bile ducts were the only original structure remaining recognizable. Sometimes there was a suggestion of a small arteriole from which the cellular reaction radiated, so that the granuloma appeared to center on an "exploded" portal arteriole (Fig. 10). Occasionally an appearance of fibrinoid degeneration of the collagen was present. When the granulomatous lesions were found in the parenchyma, there were usually an associated complete loss of liver cells (Fig. 4), and partial destruction of the reticulum. In a few instances there were focal areas of recent necrosis of still recognizable liver cells associated with an accumulation of cells like those of the fully developed granuloma (Fig. 3). In one case the liver showed well developed granulomata and also contained recent foci of necrosis. Pyknotic liver cell nuclei could be seen in these areas, and the Kupffer cells were well preserved, but there was only a minimal mononuclear cellular reaction in the necrotic areas (Fig. 2).

*Kidney.* In the kidney the granulomata were often associated with a more widespread interstitial infiltration than was usual in other organs. In many cases a generalized severe degeneration of the convoluted tubules was present. The granulomata were found more often in the cortex than in the medulla. The lesions were not so well circumscribed in the kidney as in the heart and liver, and cellular infiltration extended in an irregular manner between the glomeruli and tubules in the region of the granuloma. The most characteristic feature of the lesion as found in the kidney was the presence of recognizable fragments of renal tubules in the midst of well developed granulomatous lesions (Fig. 7). Plasma cells and especially eosinophils were generally more numerous in the kidney than in similar lesions in other organs. In one case well



developed renal granulomata were definitely peritubular in position so that the tubules were compressed and distorted, forming a nucleus of severely injured tubular cells at the center of each lesion (Fig. 9). Eosinophils were very numerous among the infiltrating cells in this case. In a few instances multinucleated giant cells of irregular shapes and of undetermined origin were found in granulomata of the kidney.

*Lung and Bronchus.* A single example of granuloma of the lung showed a necrotic granulomatous lesion in a patch of organizing pneumonia. A perivascular granuloma was present in the bronchus of the same case.

*Peritoneum.* One example of granuloma of the peritoneum due to the local application of sulfathiazole was encountered. Its most striking feature was the presence of numerous multinuclear giant cells of foreign body type.

*Bone Callus.* A single example of granuloma was found in the fibrous tissue of the callus of a simple fracture of the tibia.

### *Interstitial Inflammatory Lesions*

Interstitial inflammatory lesions were found in the heart in 6 cases, in the kidneys in 7 cases, and in the liver in 1 case, totalling 11 patients in all. As already pointed out, this interstitial inflammatory lesion sometimes blended with the borders of definite granulomata in the heart and kidneys.

*Heart.* The characteristic feature of the myocarditis in these cases was an infiltration of large mononuclear cells occurring in the fine intermuscular septa. Small and varying numbers of neutrophils and eosinophils were usually scattered among the large mononuclear cells, although in some cases eosinophils were absent. Occasionally the muscle fibers bordering these areas showed Zenker's necrosis. Only rarely did a large intermuscular septum show cellular infiltration. In those hearts with granulomatous lesions there was some adjacent interstitial infiltration of mononuclear cells, and this was associated with recent necrosis of muscle fibers (Fig. 5).

*Liver.* The interstitial inflammatory lesion of the liver has already been described as an example of hepatic necrosis. The marked interstitial inflammatory response present in this case also warrants its inclusion under this heading (Fig. 1).

*Kidney.* In the kidney the interstitial infiltration was of small extent in some cases, while in others it was very extensive. It was found in the cortex and medulla, or both. There was usually a severe nephrosis of cortical and medullary tubules. In one case (no. 4) there was a severe interstitial nephritis associated with a peculiar glomerulitis

unlike any recognized renal glomerular lesion. In contrast to the myocarditis, the predominant cells of the renal lesion were plasma cells and eosinophils. Occasionally the lesion appeared necrotic, with pyknotic nuclei in both the tubules and the cellular infiltration. In distinction to the combined interstitial and granulomatous lesions of the heart, granulomata and interstitial inflammation of the kidney were not sharply demarcated from one another.

### *Polyvascular Inflammatory Lesions*

Polyvascular lesions were found in 7 cases. The vessels involved included those of the spleen, liver, kidney, lung, air sinus, prostate, and renal pelvis. The vessels varied from the size of the afferent glomerular arterioles of the kidney to arteries of 300  $\mu$  in diameter. Typically, there was a compact, granular eosinophilic smudginess of all layers of the vessel wall with complete loss of muscle nuclei. Frequently there existed a considerable degree of separation of the necrotic elements, so that the intima bulged into the lumen. The affected vessel was surrounded by a varying depth of large mononuclear cells, a few polymorphonuclear leukocytes, and rare eosinophils. Only rarely was there a definite infiltration of inflammatory cells in the muscle layers (Figs. 11 and 12).

*Liver.* In the liver the polyvascular lesion presented itself in two forms. In one the vessel appeared to be the center of reaction. The vessel wall was necrotic and there was some adventitial and periadventitial cellular infiltration for only a short distance beyond the vessel wall. This was the less common form. The other more common expression of polyvasculitis in the liver usually involved a whole portal area in the form of a granuloma which merged with the adventitia of the necrotic portal vessels. Sometimes only remnants of an exploded vessel could be made out in the center of a granulomatous reaction (Fig. 10).

*Kidney.* Polyvasculitis of the kidney affected arteries and arterioles. The arteriolar reaction consisted of an irregular line of inflammatory cells following the course of the necrotic afferent arteriole as though being led up to the hilum of the glomerulus. Small fragments of eosinophilic material could be made out lying in this cellular infiltration, probably representing portions of necrotic vessel walls. Some of the corresponding glomeruli presented a varying degree of cellular infiltration involving varying portions of the glomerular tuft. The destruction and cellular infiltration of the glomerulus appeared to extend centrifugally from its hilus. In some cases only the hilar half of a glomerulus was affected, presenting an appearance as though the entering arteriole and proximal part of the glomerulus had been the center of a violent

explosion, while leaving the distal portion of the glomerulus untouched. In others, there was almost total disintegration of the entire tuft which was infiltrated by cells with elongated nuclei arranged in a radial pattern. The cells were again predominantly large mononuclear cells.

In both liver and kidney the lesions presented much of the appearance of the granulomata, but in the polyvascular lesion a necrotic vessel was at the center and there was more necrosis, a more acute cellular response, and less compactly arranged cells. In the spleen this polyvasculitis was sometimes part of the trabeculitis to be described.

### *Trabeculitis of the Spleen*

A peculiar necrotic and inflammatory lesion was found in the trabeculae of the spleens of 6 cases. The term trabeculitis has been coined to describe this singular inflammatory lesion. Only a few of the trabeculae in any single section were involved. They showed edema of their collagen, irregular areas of fibrinoid necrosis, and, in sections stained to demonstrate elastic tissue, an almost complete loss of the normally prominent elastica. The normal, compact, fibrillary structure of the collagen bundles was separated, producing a ragged, swollen and frayed trabecula that appeared to melt away in the pulp. Scattered through and around the involved trabeculae were large mononuclear cells. With these cells there was a variable admixture of polymorphonuclear leukocytes. In the most necrotic parts of a trabecula and along its margin abscess-like collections of polymorphonuclear leukocytes were sometimes found (Fig. 8). In one case there could be seen in the center of the most severely damaged areas necrotic arterial walls that appeared to have exploded. In this case the vascular remnants were surrounded by showers of pyknotic nuclei (Fig. 11). However, in all other cases, the changes in the trabeculae were unassociated with vessel destruction, and the lesion was present as an inflammation of the fibrous structure of the trabecula alone.

### *Nephrotic Lesion*

A pure nephrosis without inflammatory cellular infiltration was encountered in only one case (no. 22). In this case the convoluted tubules showed a marked degree of degeneration with vacuolation of cytoplasm. The nuclei of the majority of the epithelial cells of the tubules were slightly pale and swollen. A rare nucleus showed pyknosis. Bowman's spaces and the convoluted tubules were dilated and filled with flocculent eosinophilic material. The collecting tubules of cortex and medulla showed a similar dilatation, their lumina containing fuzzy precipitate or frequent hyaline casts.

### *Degenerative Changes*

Many of the cases showed a degree of parenchymatous degeneration that is not usually seen in routine autopsy material. For example, in the kidney the glomeruli often appeared larger than normal and their basement membranes swollen, while in the liver there were cloudy swelling, fatty changes, and disorganization of the liver cords. Such changes, of course, are completely nonspecific.

## DISCUSSION

### *Etiology*

In spite of the inherent difficulty of proving the cause of lesions in a morphological study of this kind, we believe that the lesions described above were produced by sulfonamide medication. This conclusion seems warranted because no other cause was found which could satisfactorily explain their development, and because sulfonamide therapy was the only factor common to all of the cases in which more or less identical lesions were encountered, as already described. Many of these lesions are identical with lesions previously described by others and attributed to sulfonamides, and many have been reproduced in experimental animals by sulfonamides.

For example, focal necrosis of the bone marrow,<sup>12, 13</sup> aplasia and immaturity of the bone marrow,<sup>14</sup> agranulocytosis,<sup>4</sup> and massive hepatic necrosis<sup>3</sup> have been recognized as sulfonamide lesions for several years. Focal necrosis of the liver with hepatitis,<sup>15</sup> interstitial myocarditis,<sup>6</sup> interstitial nephritis,<sup>16</sup> and the granulomatous foreign body reaction caused by the local application of sulfathiazole to the peritoneum<sup>17</sup> are also well established lesions. A lesion that has been less frequently reported is sulfonamide nephrosis with anuria occurring in the absence of urolithiasis medicamentosa. Prien<sup>18</sup> has discussed the pathological differences that exist between this type of nephrotic anuria and that due to the deposition of sulfonamide crystals. Recently, Rich<sup>19, 20</sup> has described cases showing lesions indistinguishable from periarteritis nodosa which he has attributed to sulfonamide medication. Among our 22 cases we found 7 with polyvascular lesions due to sulfonamides which differ in several details from those studied by Rich. We believe that the variations which we find among our own cases of sulfonamide polyvasculitis and between them and Rich's cases of sulfonamide periarteritis nodosa are to be expected, because vasculitis is a lesion found in association with many diseases as yet unrelated in etiology,<sup>21</sup> and because the lesion itself varies from disease syndrome to disease syndrome. It is this variable nature of the lesion

that has obliged us to classify it as a "polyvasculitis" rather than as a "periarteritis nodosa."

In addition to the commonly accepted sulfonamide lesions discussed above, we have encountered two unusual lesions, a granulomatous reaction and a splenic trabeculitis, that we attribute to sulfonamide medication. These peculiar granulomata are the lesions most frequently found in our series, but they have been described only recently by others. Evidence that they are sulfonamide lesions is found both in the literature and in our own material. At least 2 cases occurring in man have been described and attributed to sulfonamides,<sup>10, 11</sup> while a similar reaction has been produced in dogs with sulfadiazine.<sup>9</sup> Among our 22 cases, 13 showed granulomatous reactions involving a total of 25 organs. In 11 of these 13 cases sulfonamide lesions of the commonly recognized and accepted types were also found. The only factor common to these patients, aside from the lesions produced, was the administration of sulfonamides. No other possible etiological agent was found in a single case, and a bacterial cause was excluded in all cases by negative post-mortem blood cultures and absence of bacteria from sections of the lesions especially stained to demonstrate their presence. Moreover, the control series contained no examples of lesions similar to these sulfonamide granulomata.

So far as we are aware, the splenic trabecular lesion has not been described in the literature, although Maisel, Kubik, and Ayer<sup>10</sup> have reported a case with vasculitis of the splenic trabecular vein. They attributed this lesion to sulfonamide therapy. Six of our 22 cases showed trabeculitis, and in one of the 6 it was associated with a splenic polyvasculitis. Accepted and recognized sulfonamide lesions were also present in 5 of these 6 cases while the remaining example was associated with a peculiar nephritis that possessed many features of a sulfonamide nephritis. A careful search of the control series failed to reveal a single example of any lesion that resembled the trabeculitis of these 6 cases.

While offering evidence drawn both from the literature and from the present series of cases as support for the sulfonamides as the cause of all of the lesions described above, we are nevertheless aware of the lack of morphological specificity existing in this entire group of lesions. We are of the opinion that many etiological agents are capable of producing lesions similar to those caused by sulfonamides. This is obviously so in the case of the interstitial inflammatory<sup>22, 23</sup> and the necrotizing lesions.<sup>24, 25</sup> For example, in both scarlet fever and diphtheria an interstitial myocarditis may be found, and in the former, interstitial nephritis may occasionally occur; focal necrosis of the liver

is found in typhoid fever and many bacteria, toxins, and chemical poisons produce more or less extensive tissue death. Indeed, in our control series we have found a few examples of lesions of this character, always, however, with a demonstrable etiological agent. While the cause of polyvascular inflammatory lesions is unknown, this lesion is found in many diseases that may or may not be related in etiology and pathogenesis.<sup>21</sup> Although the control series contained no examples of the granulomatous and trabecular lesions, it is apparent that similar lesions might develop from causes other than sulfonamide treatment, such as tuberculosis, tularemia, brucellosis, and rickettsial diseases.

This manifest lack of morphological specificity of the lesions in question compels a careful analysis of each individual case in which such lesions are found before their production by sulfonamides can be established with certainty. However, if such an analysis is undertaken, there is little difficulty in establishing the presence or absence of a causal relationship for the sulfonamides, and we believe that in our own series of 22 cases such an analysis has established beyond doubt that the lesions described were caused by sulfonamide therapy.

### *Pathogenesis*

While we have established the etiological rôle of the sulfonamides for the lesions presented in this study, the pathogenesis is less easily demonstrated. However, we believe that there is adequate evidence to substantiate the hypothesis that they are of an allergic nature.

A review of the many papers dealing with sulfonamide lesions indicates that, as they have become better known, there has been an increasing tendency to regard many of them as allergic phenomena. When the drugs were first introduced, the untoward reactions and the lesions that occurred were held to be of the toxic type.<sup>1, 2</sup> Gradually, it became obvious that dosage and reaction were not closely correlated, and it was accepted that certain patients possessed an idiosyncrasy to the sulfonamides. The appearance of reactions that were obviously of an allergic nature soon indicated that idiosyncrasy alone could not account for all of the reactions and lesions that might follow therapy. Serum sickness, bronchial asthma, and angioneurotic edema exemplified this type of response.<sup>26</sup> Dermatologists were quick to recognize and warn of the sensitizing properties of sulfonamides when applied to the skin.<sup>27</sup> Wedum<sup>28</sup> obtained allergic responses in guinea-pigs, using sulfonamide azoproteins. He found a variable degree of specificity in the immunological results obtained. More recently, Leftwich<sup>29</sup> has developed an intradermal skin test for sulfonamide hypersensitivity, using the serum of patients receiving the homologous sulfonamide. He

also found that cross reactions between the various related drugs occasionally resulted. Park,<sup>30</sup> using the simple chemicals in a modified scratch test for skin sensitivity, found that certain sensitive patients would react to all of the sulfonamides tested, and even to sulfanilic acid and to procaine.

Because of the present tendency to regard many sulfonamide reactions and lesions as an expression of allergy, we have examined our series with a view to testing the validity of this conception. The evidence from this analysis strongly supports the allergic hypothesis. As we have previously detailed, 4 patients had reactions following sulfonamide therapy that were obviously of an allergic nature. These reactions included painful and swollen joints, skin rashes, conjunctivitis, and asthma. It seems significant that the lesions present in these 4 patients included every type encountered by us excepting only massive hepatic necrosis and nephrosis. This co-existence of the majority of sulfonamide lesions and sulfonamide allergy suggests that the lesions may be the morphological expression of a hypersensitivity reaction, and that this is the case whether they occur in patients who do or do not show clinical allergic sulfonamide reactions. Examination of the additional 7 cases that showed untoward clinical reactions not usually considered to be allergic in type discloses that 5 patients (nos. 8, 15, 19, 20, and 22) suffered no unfavorable reactions when first exposed to the drug. Nevertheless, when exposed to the same or a smaller amount of sulfonamide on a later occasion, they exhibited serious reactions within a very short period of time, suggesting that they had become sensitized in the interval. It is also interesting to note that of these 5 patients, 2 died of massive hepatic necrosis, and another of nephrotic anuria in the absence of renal calculi. Hitherto, these latter lesions usually have been considered to be results of toxic damage by sulfonamides. However, it is rather difficult to accept the toxic hypothesis when we consider that these patients did not react unfavorably to their first course of sulfonamide therapy, but did react immediately to a subsequent exposure, developing acute and serious sulfonamide lesions.

Others have recently considered certain sulfonamide lesions found in man as expressions of allergy. Rich<sup>19, 20</sup> has reported 2 cases of periarteritis nodosa in patients who had hypersensitivity reactions during sulfonamide therapy, and he has considered that the lesions were morphological expressions of hypersensitivity. Recently Black-Schaffer<sup>31</sup> has reported 5 cases of "anaphylactic death" following the administration of sulfonamide drugs. He described varying degrees of arterial damage and interstitial mononuclear cellular infiltrations in

these cases. He considered these lesions to be expressions of allergy and to be comparable to the experimental lesions produced by sensitivity reactions to proteins.

It is true also that many of the lesions that have been described in this paper are comparable to the lesions produced by hypersensitivity reactions to foreign protein in experimental animals. For example, Longcope<sup>32-35</sup> produced interstitial and focal inflammatory reactions in the heart, liver, and kidneys of rabbits, dogs, and guinea-pigs by repeated injections of foreign proteins. The parenchymatous degeneration and predominant mononuclear response of these lesions bear a similarity to the lesions we have found. More recently, Klinge,<sup>36</sup> Vaubel,<sup>37</sup> and Knepper and Waaler<sup>38</sup> have produced arteritis and collagenous and parenchymatous degeneration in sensitized rabbits by repeated injections of foreign protein. More striking, however, was their production of unequivocal granulomata in skeletal and heart muscle and in collagenous tissue, centering on blood vessels. Fundamentally these lesions consisted of focal degeneration of the tissues involved with a histocytic monocytic response. Working with monkeys in somewhat similar experiments, Ferraro<sup>39</sup> has obtained vasculitis and granulomatous formation in the central nervous system.

Rich<sup>19, 20</sup> and Rich and Gregory<sup>40</sup> have ascribed experimental and clinical periarteritis nodosa to hypersensitivity to foreign protein and/or sulfonamide, and have concluded that periarteritis nodosa may be an expression of allergy caused by widely differing antigens. Their work provides additional confirmation for the allergic nature of the polyvasculitis we have described. Of more interest is their experimental production of foci of collagenous necrosis and mononuclear cellular infiltration in the heart by hypersensitivity reactions to foreign protein.<sup>41</sup> This lesion is strictly comparable to the collagenous necrosis and cellular infiltration of the trabeculae of the spleens in 6 of our cases. Clark and Kaplan<sup>42</sup> have also described necrotizing arteritis and periarteritis in small coronary arteries, as well as subendocardial proliferations of histiocytes in the hearts of 2 patients who died while suffering from serum disease. They interpreted these lesions as expressions of hyperergy following the administration of foreign serum.

In presenting the above discussion we have sought to show that there is ample justification for the conception of the allergic nature of the sulfonamide lesions. It has been noted that various investigators have demonstrated that the sulfonamides can produce immunological reactions *in vivo*. The occurrence of significant allergic manifestations, both in the present series of cases and in the cases of sulfonamide lesions that others have reported, has been discussed, and an attempt



has been made to show that an acceptable degree of morphological similarity exists between the lesions produced by hypersensitivity to foreign protein and those attributable to sulfonamides. We conclude, therefore, that the lesions reported in this paper are based upon sulfonamide hypersensitization.

The fundamental pathological change that is elicited by the allergic reaction is not apparent in the material of this series. There is no evidence that any single tissue is the primary reactant. Basically, all of the lesions we have examined are a complex of tissue destruction and of proliferation of the reticulo-endothelial cells in the affected area. For example, the infiltration and the granulomata of the heart seem to be fundamentally an expression of the destruction of tissue with an associated mononuclear cell response. Similarly, in the liver the areas of early necrosis with a minimal or moderate mononuclear cell response seem to be merely the initial stages in the development of the mature granuloma in which none of the original parenchyma remains. Furthermore, the massive hepatic necrosis and the case of nephrosis appear to be examples of massive parenchymatous destruction resulting in death before a mononuclear cell response is apparent. But, it is to be remembered that the preservation of the Kupffer cells in the midst of extensive hepatic necrosis, and the prominent reticulo-endothelial proliferation found in all lesions, suggest that while degeneration and necrosis are the earliest observable morphological changes, they are not necessarily the fundamental ones. We cannot determine, therefore, whether necrosis or reticulo-endothelial activity is cause or effect of the reaction, but, regardless of the varying proportions found between these two elements, it is our conviction that all of the sulfonamide lesions described are fundamentally the same, differing only in the phase and intensity of the reaction.

Whatever the fundamental reaction elicited by the allergy may be, it is impossible to say exactly what factors permit its development in some persons and not in others. In most of the cases in the present series the patients were suffering from an illness, more or less serious, before sulfonamide drugs were given. There is some evidence to suggest that altered metabolism from disease or nutritional deficiency may play an important rôle in the development of sulfonamide sensitivity. This probability is borne out by a recent study in which 5,000 healthy men received sulfadiazine prophylactically with negligible untoward effects.<sup>43</sup>

#### SUMMARY AND CONCLUSIONS

Examination of 375 autopsies of patients who had received sulfonamides revealed 22 cases with lesions attributable to sulfonamide medication. These lesions were regarded as severe enough to cause

death in 7 cases, were major factors contributing to death in 7 additional cases, and were apparently of negligible importance in the remaining 8 cases. No statistical interpretation of these figures is possible. Among these 22 cases examples of the majority of the commonly reported sulfonamide lesions were found. In addition to these, however, a granulomatous reaction that has been reported but rarely was found to be the lesion of highest incidence, occurring in 13 cases. A unique lesion, a splenic trabecular necrosis and inflammation, was found in 6 cases. The evidence that these latter lesions were caused by sulfonamide therapy consists of the lack of other demonstrable causes, their co-existence with recognized sulfonamide lesions and, in the case of the granulomata, their experimental production with sulfadiazine. It was found that all lesions invariably combined necrosis of the tissues involved with activity of the reticulo-endothelial system. This basic similarity of the structural alterations indicated a fundamental pathogenesis common to all of the lesions. The association of all types of lesions described with clinical evidence of sulfonamide hypersensitivity, and the essential identity of these lesions with those produced in animals, by various investigators, by foreign protein sensitization, led to the conclusion that the lesions were always an expression of allergy.

While this report is obviously of some interest to the clinician and therapist, we believe that its chief value lies in its relation to the general problem of the pathology of allergy in man, an important and, as yet, relatively unexplored field.

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[ Illustrations follow ]

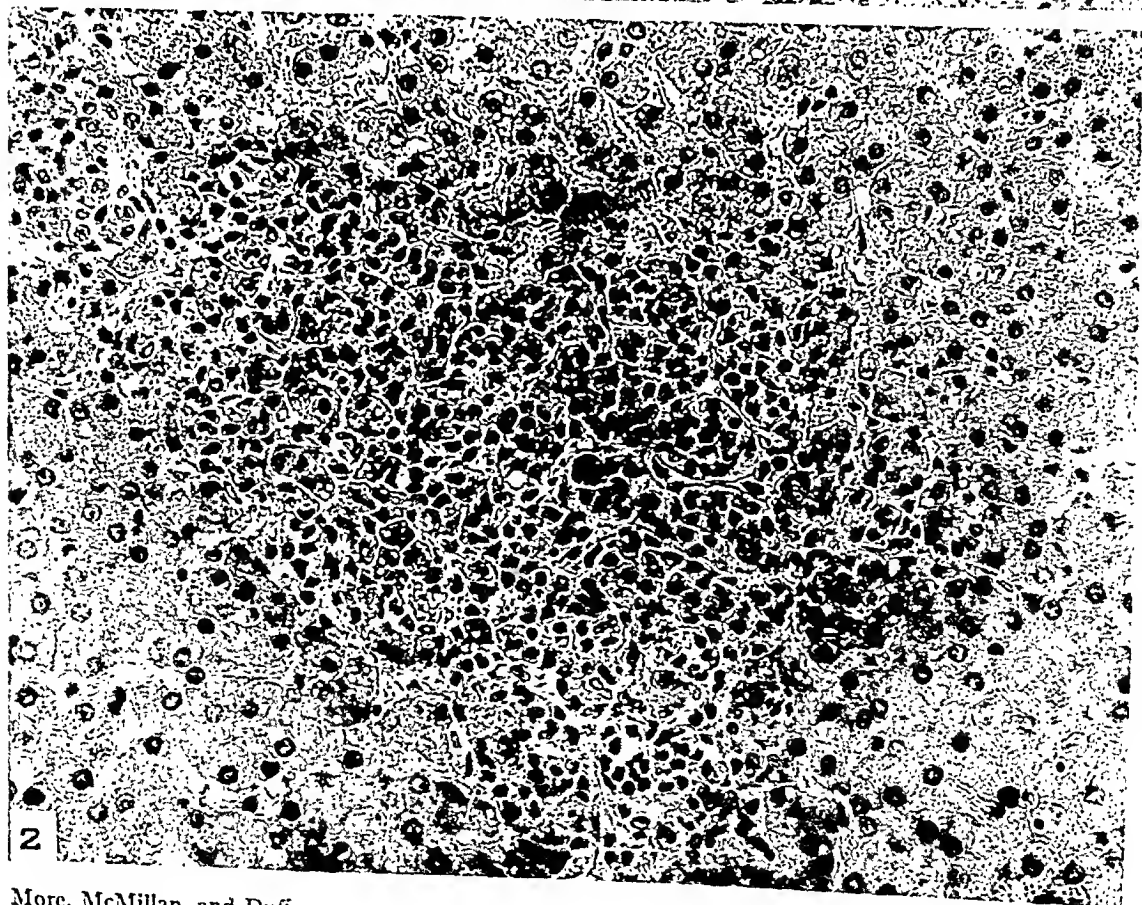
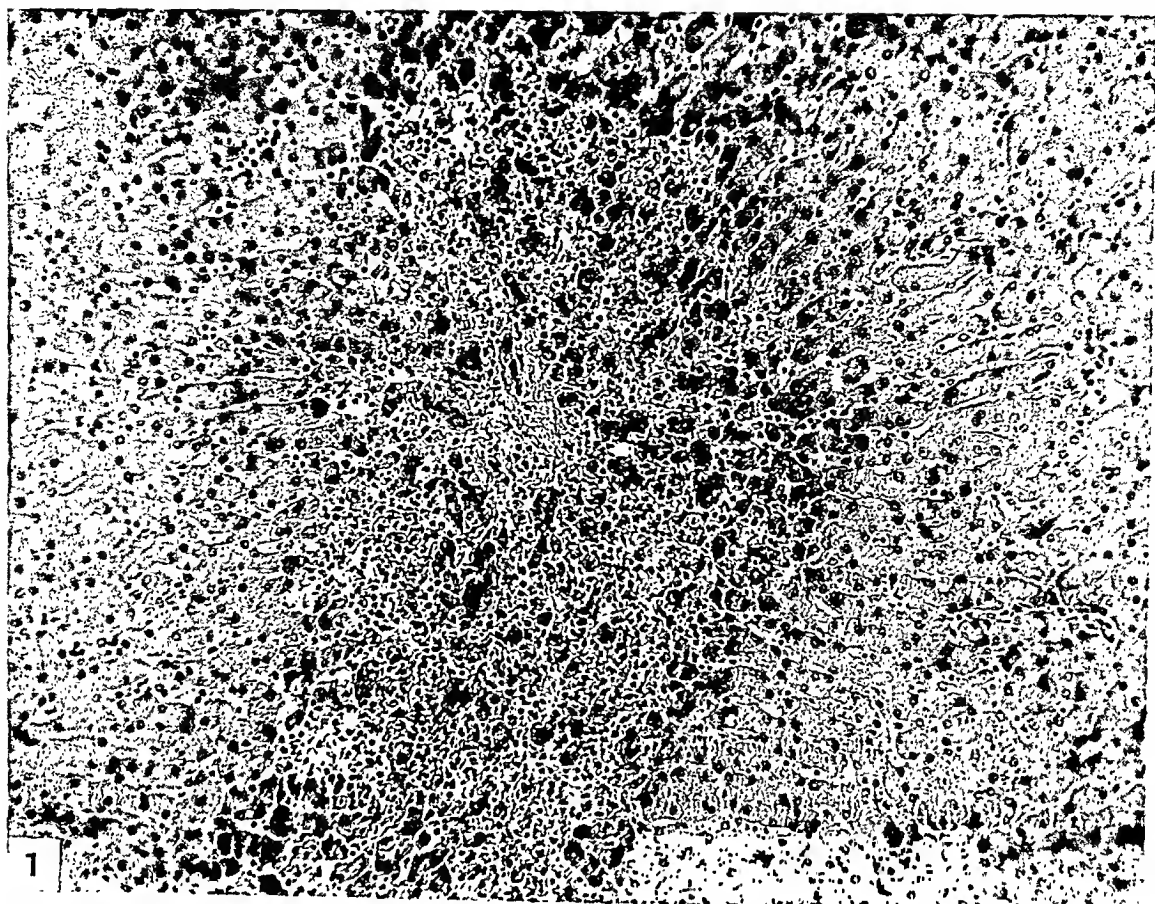
## DESCRIPTION OF PLATES

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### PLATE 138

FIG. 1. Case 9, liver. An area of recent central necrosis with disruption of the liver cords is seen. An interstitial infiltration of inflammatory cells which are predominantly mononuclear accompanies the necrotizing reaction. The liver cells bordering the immediate area of necrosis show severe degeneration, while those that are slightly removed show only cloudy swelling. Hematoxylin and eosin stain.  $\times 137$ .

FIG. 2. Case 16, liver. Many areas of focal necrosis similar to the one in this field were found in this liver, which also contained mature granulomatous lesions. The size and shape of the lesion, in addition to the appearance of the liver cells, suggest that this lesion is the precursor of the typical granulomatous reaction (Figs. 3 and 4). It is to be noted that the characteristic cellular reaction of the granuloma can be identified in this lesion, but that it is minimal. Hematoxylin and eosin stain.  $\times 310$ .



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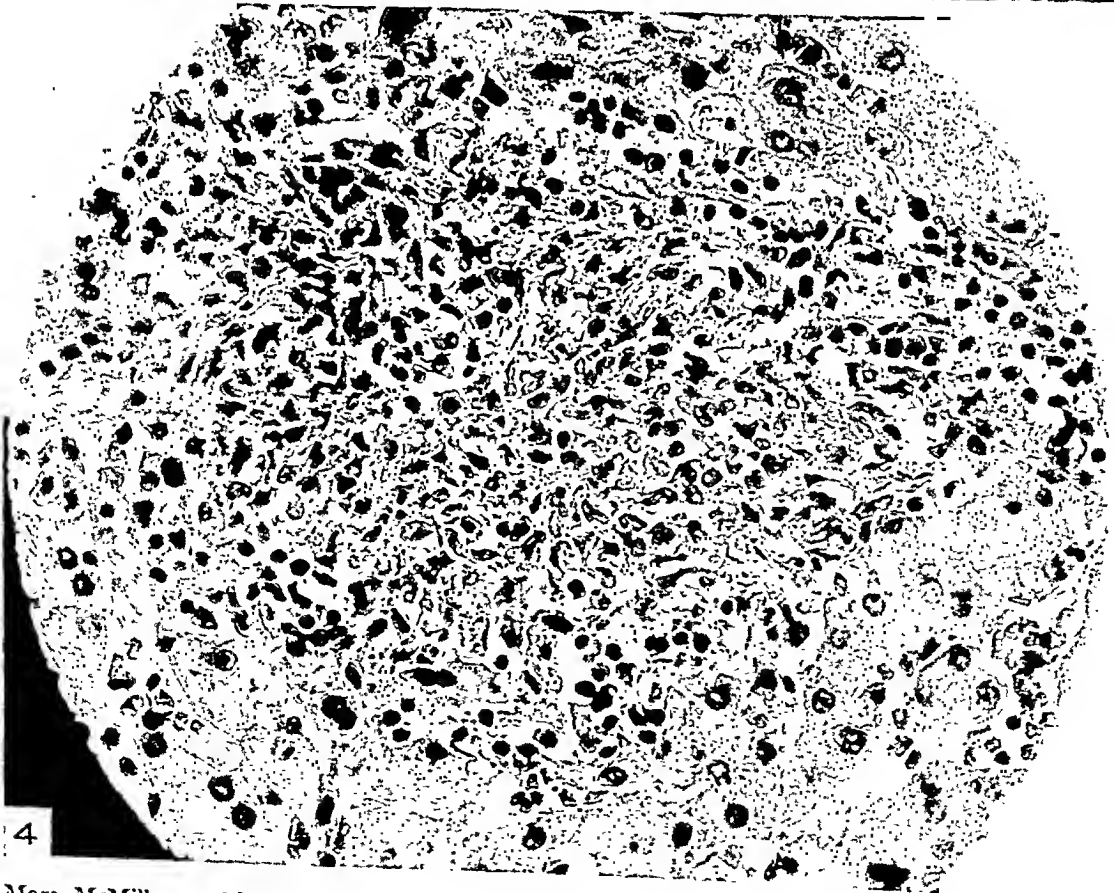
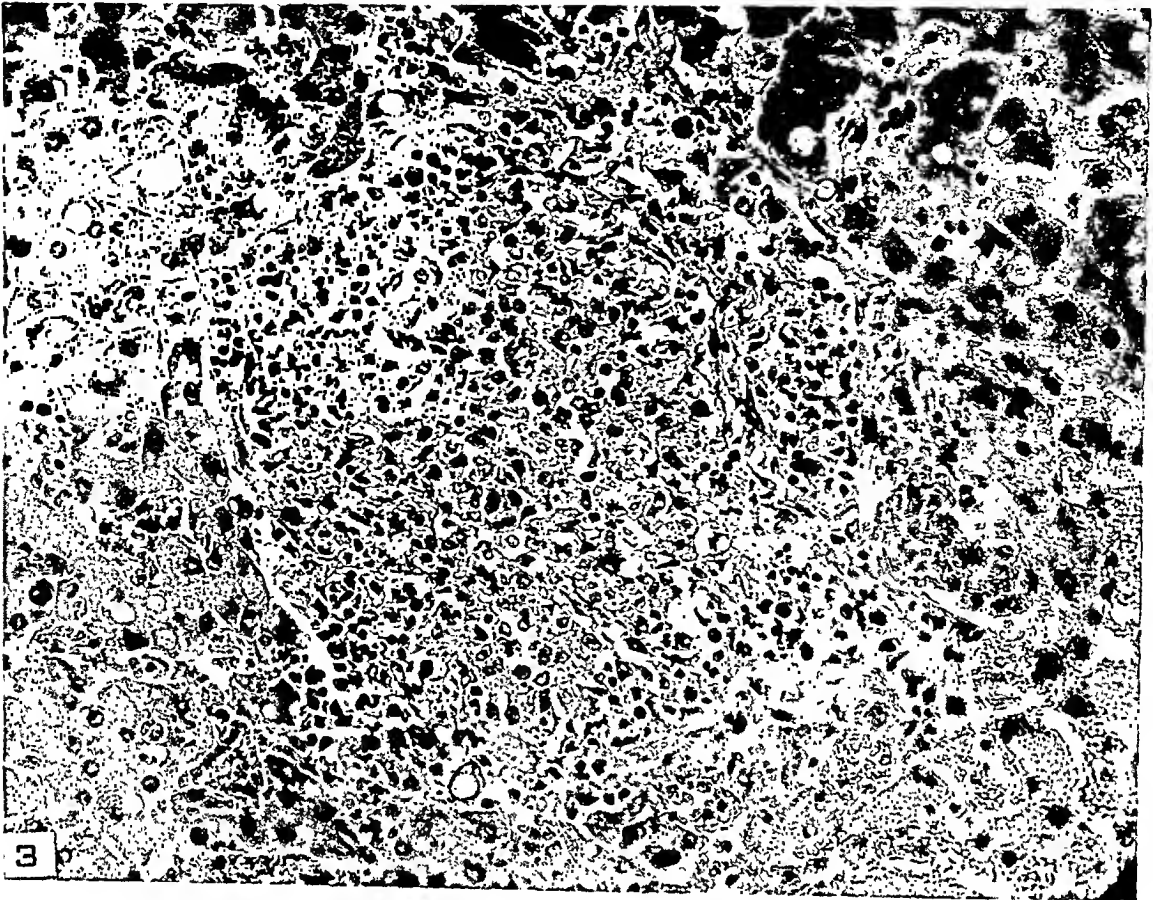
Sulfonamide Allergy in Man

PLATE 139

FIG. 3. Case 21, liver. A moderately mature granuloma of the liver is seen to show very few identifiable liver cells among the cells of the monocytic reaction. Plasmotoid and polymorphonuclear leukocytes are present in small numbers. (See also Figs. 5 and 6 from the same patient.) Hematoxylin and eosin stain.  $\times 260$ .

FIG. 4. Case 14, liver. At a high magnification, a mature granuloma of the liver shows the characteristic tessellated appearance of the lesion. The predominantly monocytic nature of the cellular reaction and the blurred eosinophilic interstitial material can be identified. No liver cells are found in this lesion. The lesion is clearly demarcated from the neighboring liver cells. Hematoxylin and eosin stain.  $\times 415$ .





More, McMillan, and Duff

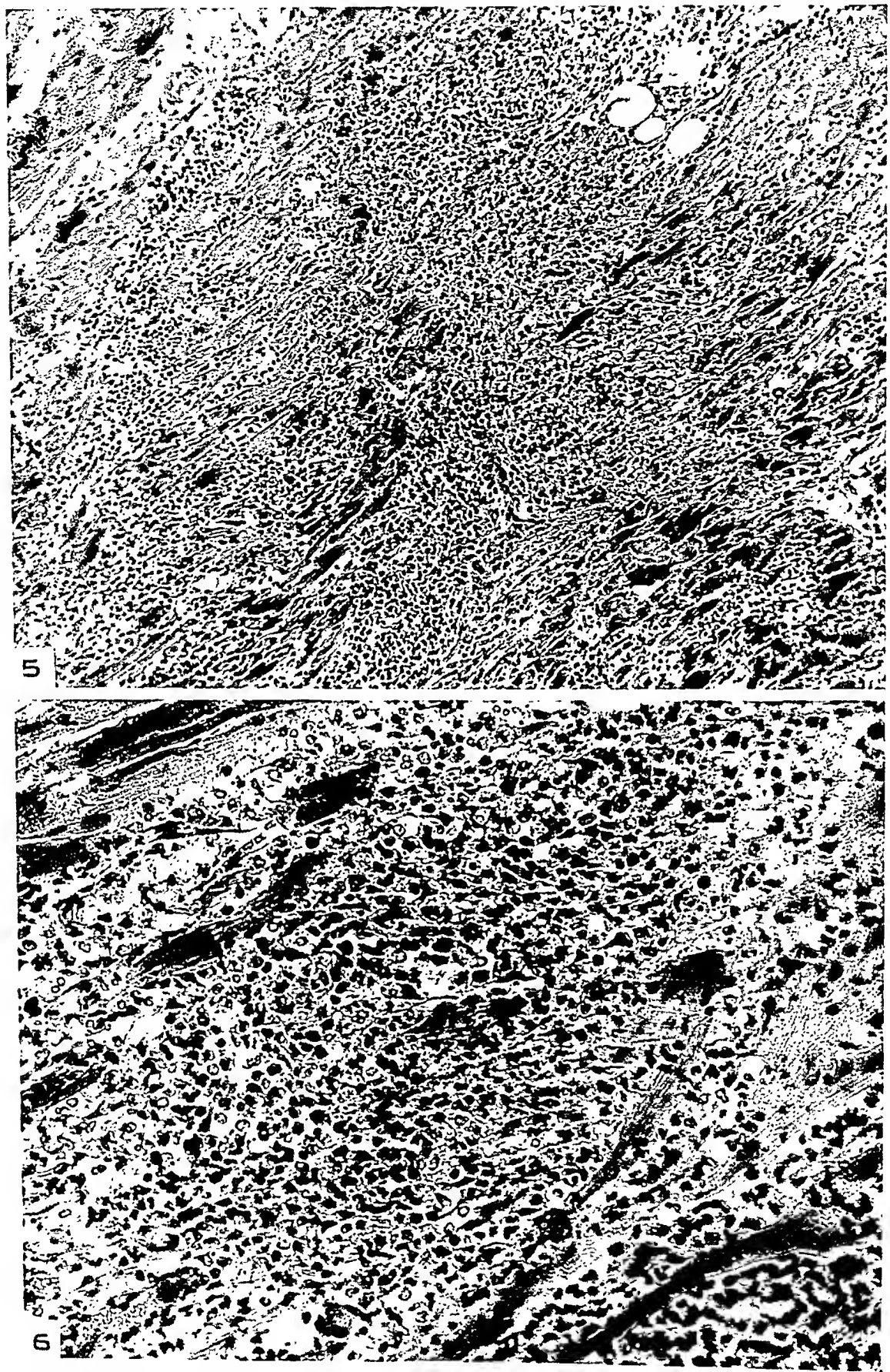
Sulfonamide Allergy in Man



PLATE 140

FIG. 5. Case 21, heart. At a low magnification, a lesion from the same section of heart muscle as Figure 6 shows a rather mature and widespread granulomatous lesion. The same features that may be noted in Figure 6 are present, with the addition of an extensive associated interstitial myocarditis. Lesions of this extent were numerous in all parts of the myocardium in this case. (See also Figs. 3 and 6 from the same patient.) Hematoxylin and eosin stain.  $\times 124$ .

FIG. 6. Case 21, heart. A moderately mature granulomatous focus is seen lying in the heart muscle. The muscle fibers close to the lesion show necrosis of a Zenker's type. The sharply demarcated nature of the muscle destruction is striking. The cellular content of the granuloma is of a rather uniform mononuclear type, but plasmotoid and polymorphonuclear cells are numerous. (See also Figs. 3 and 5 from the same patient.) Hematoxylin and eosin stain.  $\times 314$ .



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- FIG. 7. Case 12, kidney. A mature granuloma with rather ill-defined limits is seen surrounding necrotic, but still recognizable, renal tubules. There is a slight interstitial nephritis. Hematoxylin and eosin stain.  $\times 150$ .
- FIG. 8. Case 15, spleen. A swollen, ragged and frayed splenic trabecula is seen. It stains poorly and its few nuclei are pyknotic. It is heavily infiltrated with inflammatory cells, some of which form abscess-like foci at the border between the pulp and the trabecula. (See also Fig. 10 from the same patient.) Hematoxylin and eosin stain.  $\times 106$ .
- FIG. 9. Case 13, kidney. Peritubular granulomatous foci are seen about the collecting tubules near the tip of a renal pyramid. Necrotic tubular epithelium can be identified with difficulty. Hyaline casts and a multinucleated, crescentic giant cell are to be noted. Hematoxylin and eosin stain.  $\times 144$ .

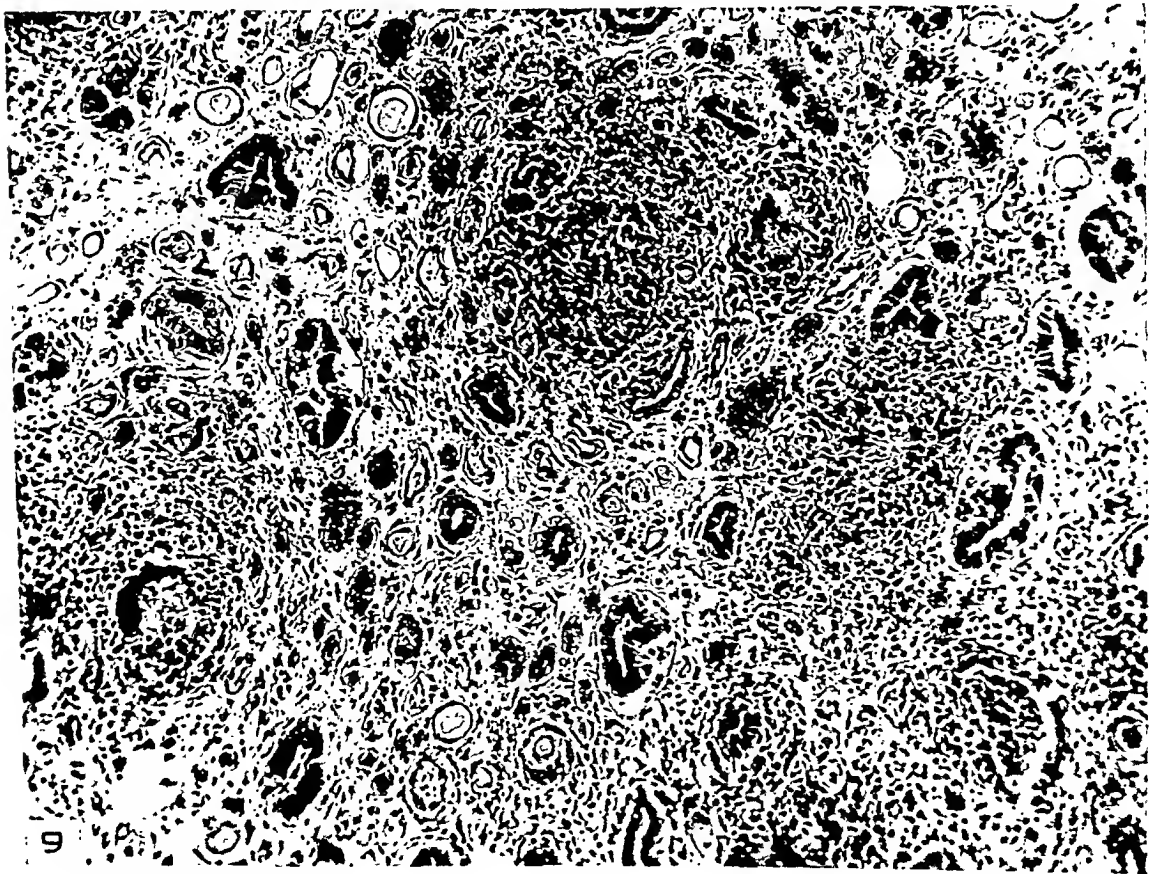
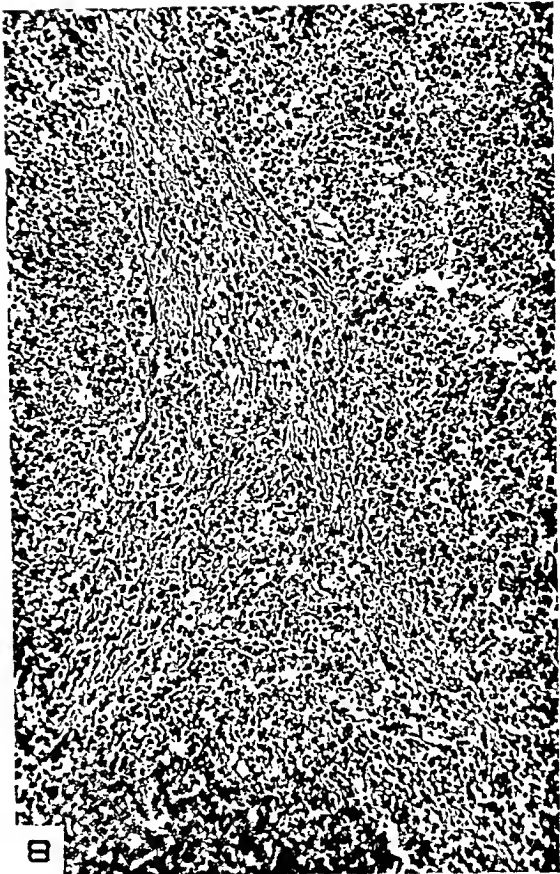
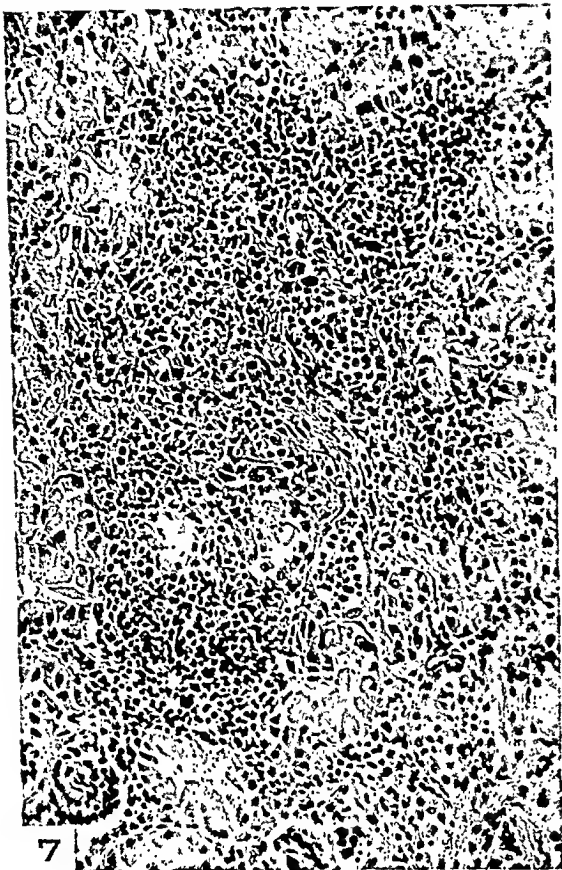
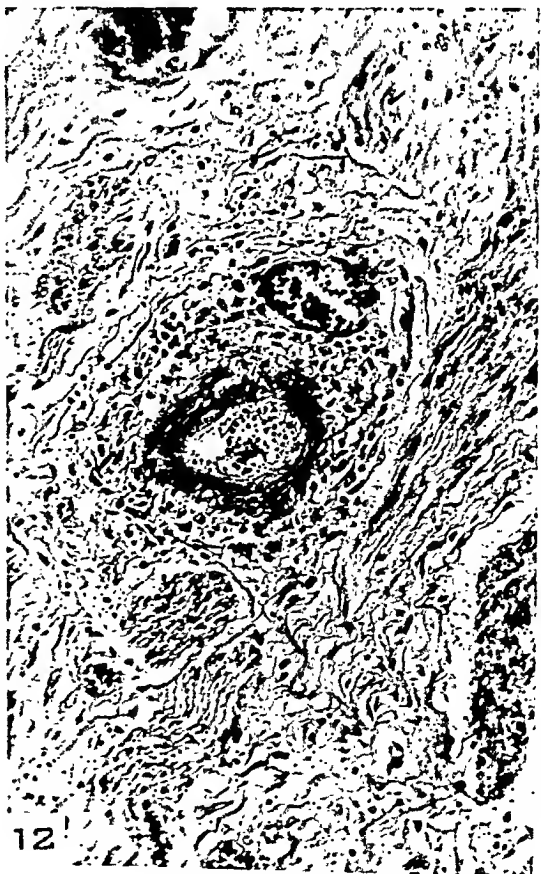
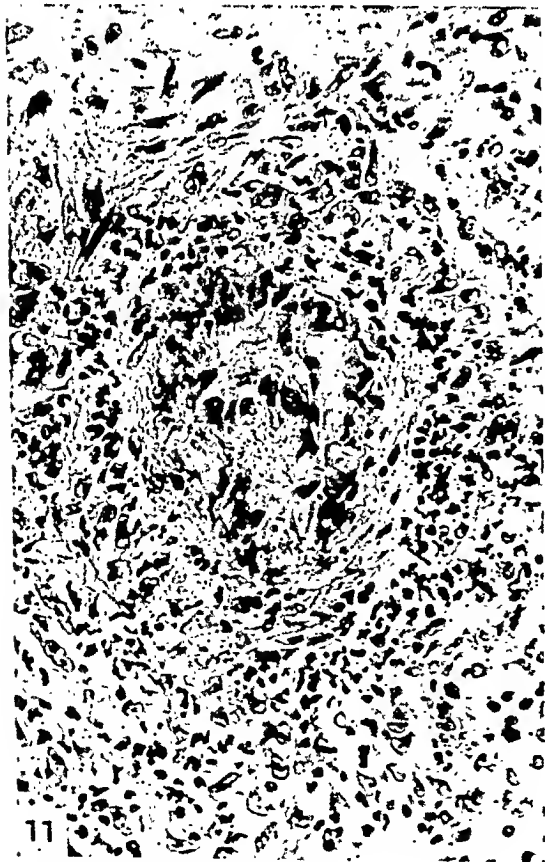
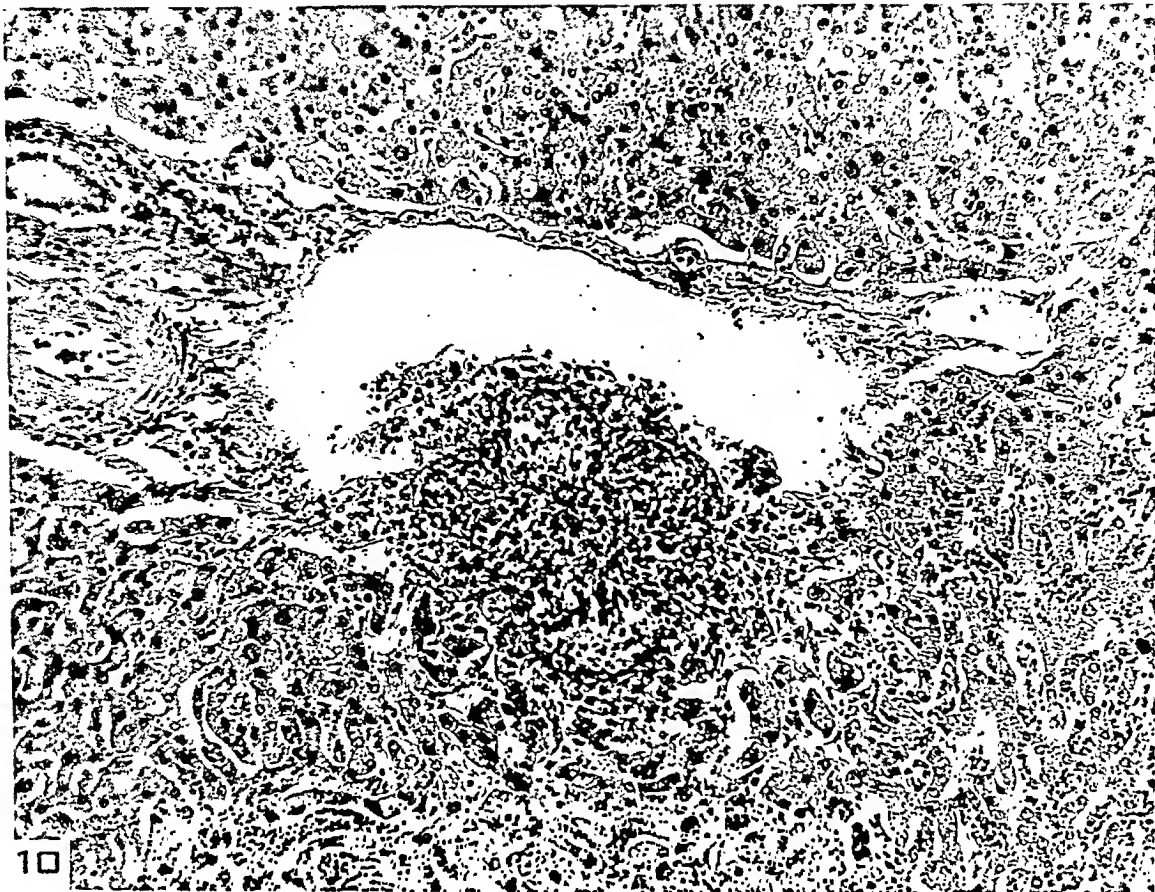


PLATE 142

FIG. 10. Case 15, liver. A rather acute granulomatous reaction is seen involving a portal area. The lesion has an explosive appearance. There is destruction of a segment of the wall of a portal vessel and the lesion contains fragments of material that suggest fibrinoid necrosis of collagen. The general liver parenchyma shows engorgement and cloudy swelling. (See also Fig. 8 from the same patient.) Hematoxylin and eosin stain.  $\times 146$ .

FIG. 11. Case 4, spleen. There is vasculitis of a medium-sized trabecular artery. It shows complete destruction with fibrinoid necrosis of the media, and plugging of the lumen with desquamated, proliferating intimal cells. The adventitia is infiltrated with mononuclear cells admixed with substantial numbers of polymorphonuclear leukocytes and small round cells. The cellular reaction extends into the trabecula. Hematoxylin and eosin stain.  $\times 340$ .

FIG. 12. Case 17, pelvis of ureter. Vasculitis affecting a small artery and vein is seen. The musculature of the artery is smudgy, necrotic, and eosinophilic in staining reaction. It possesses no nuclei. The adventitia and periadventitia are infiltrated with small round cells and polymorphonuclear leukocytes. Hematoxylin and eosin stain.  $\times 160$ .







## EXPERIMENTS WITH JAAGSIEKTE\*

NIELS DUNGAL, M.D.

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In 1933, a disease of sheep was introduced into Iceland from Germany which has caused enormous losses to the farmers, who in some places have lost 60 per cent and even more of the stock during a couple of years, as mentioned in a previous publication.<sup>1</sup> The identification of this disease with jaagsiekte, as described by Cowdry<sup>2,3</sup> and Cowdry and Marsh,<sup>4</sup> Mitchell,<sup>5</sup> and de Kock,<sup>6,7</sup> seems to be justified, as described by me<sup>8,9</sup> in previous publications. As symptoms and pathological changes have been described in the publications already referred to, I shall only repeat here what is necessary for a better understanding of my experimental work.

The characteristic *symptoms* are a slowly increasing dyspnea accompanied by excretion of frothy, slightly opalescent, watery mucus from the respiratory passages. The beginning of the disease is insidious and 6 months usually pass from the time of contact until visible symptoms appear. There is no elevation of temperature. The most reliable clinical test in suspected cases is to turn the sheep with head downwards and notice whether watery fluid drips from the nose. If it does it is a reliable symptom, which has never failed in diagnosing this disease. On the other hand, this secretion may be absent in the initial stage and also in the late stages of very chronic cases, in which fibrosis has superseded the specific changes.

### PATHOLOGICAL CHANGES

The initial changes, which are strictly limited to the lungs, consist of tiny grayish spots, just visible to the naked eye, which soon tend to become confluent. In sections they appear with low magnification (Figs. 1 and 2) as dispersed, darker colored spots of varying size, some being tiny tufts, others displaying distinct adenomatous characteristics. Some can be traced to bronchial epithelium (Fig. 3), others to the alveolar lining (Fig. 4).

A constant feature is a more or less pronounced cellular exudate in the alveoli, consisting chiefly of mononuclear cells, which are derived at least partly from the lining epithelium, but segmented neutrophilic leukocytes are frequently seen (Fig. 3) and red blood corpuscles are often conspicuous. In rare cases a pronounced lymphoid hyperplasia may be observed (Fig. 5). In some cases intrabronchial proliferation is very pronounced (Figs. 5 and 6), but is always limited to the

\* Received for publication, July 14, 1945.



bronchioles. I have never observed any growth in the bronchi visible to the naked eye.

The structure of the epithelium is, as a rule, very regular and of the type of a benign adenoma (Figs. 7 and 8), but in exceptional cases the structure may assume a more irregular aspect (Fig. 9), suggesting malignant growth. Yet I have never seen metastatic growth in the tracheobronchial glands or elsewhere.

In advanced cases the lungs become greatly enlarged (Fig. 10), mottled grayish blue (Fig. 11), smooth and tense. A frothy watery liquid oozes from the cut surface, displaying a nodular, friable, light grayish tissue (Fig. 12) from which a viscid grayish mucus exudes on pressure, not unlike "cancer milk" except that it is gray instead of white. The edematous accumulation in the lungs is sometimes enormous, and when the lungs are enlarged and distended with liquid the increase in weight may be pronounced. Frequently I have seen lungs weighing 2500 to 3000 gm. the pair, whereas normal lungs rarely weigh more than 400 gm. A pronounced hypertrophy of the heart may be present in such cases, but as a rule it is not conspicuous. Sudden death is sometimes observed as a consequence of increased exertion, as, for instance, when a sick sheep is made to run; but usually terminal pneumonia leads to death.

### EXPERIMENTS

In spite of innumerable attempts to cultivate the causative organism, all such attempts have failed. As a rule no growth has been observed. Various strains of streptococci, which were isolated from many cases and injected into healthy sheep, evidently bore no etiological relationship to the disease.

Small pieces of tissue, up to pea size, cut with sterile precautions from the specific tissue and put directly into broth, gave no growth after a week's incubation in some cases where contaminating organisms were not present.

A natural infection has never been observed in cattle, although sick sheep have frequently been housed in cow stalls. Goats have not been affected on the few farms where they were kept with sick sheep. Neither have we ever encountered such a disease in man in our autopsies, and there is no case on record in this country which suggests a possible transfer of jaagsiekte to man.

Experimental inoculations were made on mice, guinea-pigs, and rabbits, but all without any positive results. I therefore limited my experimental inoculations to sheep as the only animals susceptible to this disease. Previous experiments<sup>8</sup> had shown that the disease can easily

be transmitted by housing healthy sheep with sick ones. When sheep were housed together, several means of transmission were possible: (1) through feces or urine, (2) through external parasites, (3) through bronchial secretion dispersed in the respiratory air.

1. Three lambs were fed by stomach tube with feces from a sick sheep. After 8 months they were killed, and no traces of adenomatosis were found nor any particular changes in the lungs.

2. The possibility that the wool-louse, *Melophagus ovinus*, might convey the virus was considered, and for that purpose a great number of melophagi from sick sheep were transferred to 5 healthy lambs. These lambs were killed after 8 to 10 months, with negative result.

In order to exclude any possibility of infection except the respiratory air, the following experiment was made:

Two healthy young sheep were confined (March 18, 1941) with a sick sheep in a small compartment in such a way that the head and neck of the sick sheep were directed to the outside through a hole in the wall. The rim of the hole was padded, so that no current of air could pass through. In this way the whole body of the sick sheep, with the exception of the head, was in contact with the healthy sheep which, in the narrow compartment, turned their heads in the opposite direction. An infection might be supposed to take place if any external parasite or fecal material were contagious. The sick sheep was held in this position for 14 days, when it was taken away by removing the wall. The 2 healthy lambs were kept for a month afterwards in the same compartment and then taken to another compartment where they had more space. Four and a half months later one of the lambs died suddenly of pneumonia. Autopsy showed no sign of jaagsiekte. The other lamb remained healthy and showed no signs of jaagsiekte when killed 7 months after the start of the experiment.

3. The previous experiments having pointed to an exclusion of all but the respiratory factor, the following experiments were made:

Two lambs were put in an elevated compartment 1.5 yards above the head of a sick sheep kept in a lower compartment. Strict precautions were taken that no material particles and nothing except the air could move from the lower to the upper compartment. This experiment was repeated three times, each trial lasting 4 to 6 months. Of 8 lambs used in these experiments, 3 contracted typical jaagsiekte, but 5 showed no signs of the disease.

A further attempt to demonstrate the respiratory transmission of the disease was the following:

On January 28, 1941, a sick sheep was made to breathe through a 20 per cent solution of glycerine in saline water for 30 minutes. This

breathing was made possible by employing a specially constructed mask with two valves, one for expiration and one for inspiration, the expiratory valve being connected with rubber tubing to the bottom of a jar containing the glycerine mixture. After the sheep had breathed through this mixture, the slightly opalescent fluid was injected, 5 cc. intratracheally and 2 cc. intrapulmonally on the right side, into each of 3 lambs. Two lambs developed typical jaagsiekte, one of them (no. 873) showing clinical symptoms 4 months later when extensive typical lesions were found. In the other (no. 872) a small pea-sized nodule was found in the middle lobe of the right lung, histologically typical adenomatous jaagsiekte. This node was cut in serial sections in its entirety and every other section examined. No worms, eggs, or larvae could be found in it or its vicinity.

When lamb 873 showed unmistakable symptoms of jaagsiekte, it was made to breathe through a glycerine-saline solution as described. This fluid was filtered through a gradocol membrane with pores  $0.9 \mu$  in diameter. While the filtration was taking place the lamb was killed to corroborate the clinical diagnosis. A frozen section showed a typical histological picture of jaagsiekte. Two cc. of this filtrate was injected into the right lung of each of 4 lambs on May 28, 1941. Four months later (September 20, 1941) one of these lambs showed symptoms of jaagsiekte. It was killed and both lungs were found to be affected. The main lesion was located in the right apical lobe, half of which was partly indurated, partly soft and friable, light gray and nodulous, macroscopically typical of jaagsiekte. Histologically the lesions were also typical: papillomatous adenomatous lesions with surrounding mononuclear alveolar exudation. No bacteria were visible in direct preparations. The other 3 lambs were killed later on the same day. In the right lung of one of them (no. 877) a chronic pneumonia was found as an area of slight consolidation on the posterior part of the posterior lobe. Papillary epithelial proliferation was seen in some places, but it was so slight that a positive diagnosis of jaagsiekte was not made. The other 2 lambs were normal.

Parallel with the first experiment, started January 28, 1941, another was begun with the filtered expiratory fluid. The filtration was done through a  $0.9 \mu$  gradocol membrane. Of this filtrate, 5 cc. was injected intratracheally into each of 4 lambs.

Four months later (May 26) one of these lambs, which, as always, were kept isolated from other sheep, was suspiciously dyspneic. It was killed the following day and the autopsy showed a typical picture of jaagsiekte in both lungs. The whole apical lobe was consolidated, fairly dense in the upper half, but soft, friable and almost gelatinous in the

lower portion where the process was in progress. In the right lower lobe typical scattered lesions were found. The left lung also showed fairly extensive lesions with friable, soft tissue. Histologically these lesions were typically adenomatous. The other 3 lambs were killed on the same day, and none of them were found affected.

*Experiments with Filtered and Unfiltered Material from Affected Lungs*

Experimental inoculations with filtered and unfiltered tissue were very discouraging. As we had before our eyes the constant spread of the disease among the flocks, I was surprised at the negative results with inoculations of affected lung tissue. These experiments were conducted along the same principal lines: Typical jaagsiekte-lung tissue from freshly discovered cases was ground with sterile sand in a mortar with saline solution. This was filtered through eight layers of gauze and injected directly when unfiltered material was used, or filtered through Chamberland, Berkefeld, or Seitz filters or gradocol membranes when filtered material was used.

Tables I to III give a survey of these experiments.

*Experiments with Collodion Sacs*

After a preliminary study, it was found easy to make transpleural operations on lambs for the purpose of inserting material into their lungs. Under ether narcosis a rib was resected and the incision in the pleura plugged immediately by pulling the lung tissue outwards through the wound to prevent pneumothorax. In this way small collodion sacs

TABLE I  
*Experiments with Unfiltered Material*

Experiment started	Mode of injection	Duration of experiment	Results	
			Positive	Negative
		(months)		
March 6, 1937	Injected intrapulmonally into 3 lambs	7½	1	2
May 3, 1937	Injected intrapulmonally into 3 lambs	7½	1	2
Nov. 12, 1937	Injected subcutaneously into 2 lambs	6	0	2
May 4, 1938	Injected intrapulmonally into 5 lambs	6	0	5
Oct. 3, 1938	Injected intravenously into 3 lambs	3	0	3
Nov. 18, 1938	Injected intrapulmonally into 5 lambs	2-3	0	5
Sept. 16, 1939	Injected intrapulmonally into 3 lambs	2-8	0	3
April-May, 1939	Injected intrapulmonally into 5 lambs	3-4	0	5
Sept. 22, 1939	Injected intrapulmonally into 3 lambs	2-9	0	3
Sept. 16, 1939	Sprayed into nostrils of 3 lambs	2-8	0	3
Jan. 26, 1940	Injected trypsin-digested, unfiltered tissue into 3 lambs, also sprayed into nostrils	6	0	3
Totals			2	36

containing finely minced jaagsiekte-lung tissue could be inserted into the lung, whereupon the incision in the lung was sutured and the pleural wound closed. After the skin wound had been sutured and the lambs awakened from their narcosis, they were brisk and walked about as before.

On January 4, 1939, 5 lambs (nos. 700 to 704) were operated upon in this way, one collodion sac with jaagsiekte tissue being inserted into

TABLE II  
*Experiments with Filtered Material*

Experiment started	Filter employed	Mode of injection	Number of lambs	Duration of experiment	Results	
					Positive	Negative
1937:				(months)		
March 11	Seitz E. K.	Intrapulmonal	5	7½	0	5
Nov. 12	Seitz E. K.	Intrapleural (1 died 2 weeks later from inter-current infection)	2	8½	1	1
Nov. 12	Chamberland L 2	Intrapleural	2	8½	1	1
Nov. 12	Seitz E. K.	Subcutaneous	3	8½	2	1
1938:						
May 4	Berkefeld-N.	Intrapulmonal	5	5	1	4
Sept. 5	Seitz E. K.	Subcutaneous	10	½-3½*	0	10
Sept. 15	Seitz E. K.	Intrapulmonal	10	½-4½*	0	10
Oct. 3	Seitz E. K.	Intravenous	10	4	0	10
Oct. 3	Seitz E. K.	Intrapulmonal	5	4	0	5
1939:						
Sept. 16	Chamberland L 2	Intrapulmonal	3	7	1	2
Sept. 16	Chamberland L 2	Sprayed into nostrils	3	4-9	0	3
Sept. 22	Chamberland L 2	Intrapulmonal	3	8	0	3
1940:						
Aug. 23	Chamberland L 2	Intrapulmonal	3	8	0	3
Totals			64		6	58

\* These lambs began to die from diarrhea 2 weeks after the experiment was started.

the right lung of each. From January 10, lamb 701 was dyspneic, with fever, and it was killed on January 16. An extensive pneumonia was found in both lungs, but nothing resembling jaagsiekte. The operative wound was found completely healed with no reaction around it. The pneumonia was found to be caused by the bacillus of contagious pneumonia, yielding unusually hemolytic colonies.

On January 28, lamb 700 was killed, after showing conspicuous dyspnea. Nothing was found at autopsy except a slight atelectasis about the sac, around which a whitish fibrous capsule had formed. Nothing resembling jaagsiekte was found macroscopically or microscopically.

On March 4, lamb 702 was killed. No changes were found in the lungs.

No. 703 was killed on May 13, almost 4 months after insertion of the sac, without having shown any suspicious symptoms. The lungs were of normal size and displayed no outward abnormality. The colodion sac was found intact, but hardened and embedded in a fibrous capsule. Around this capsule was a patchy tissue, 1 to 1.5 cm. in thickness, with grayish nodules, greatly resembling jaagsiekte. Histologically this tissue was found to be typical adenomatous jaagsiekte.

No. 704 was killed on April 11 and nothing abnormal found.

TABLE III  
*Experiments with Injection of Bronchial Secretion*

Experiment started	Unfiltered material				
	Mode of injection	Number of animals	Duration of experiment (months)	Results	
				Positive	Negative
Sept. 8, 1937	Sprayed into nostrils	2	4½-7	0	2
April 30, 1937	Intratracheal	5	3½	0	5
Oct. 3, 1938	Intrapulmonal, directly from a sick sheep	3	3½	0	3
Oct. 3, 1938	Intratracheal, directly from a sick sheep	2	3½	0	2
	Totals	12		0	12
Filtered material					
Sept. 6, 1937	Chamberland L 3, sprayed into nostrils	2	8	1	1
April 30, 1938	Berkefeld-N, intratracheally	5	4	0	5
	Totals	7		1	6

Of the 5 lambs in this experiment, only one developed jaagsiekte, but it must be remembered that 2 of the lambs died within a month of the inoculation and the other 2 were killed rather early, within 3 months after the inoculation.

### *Transfusion Experiments*

Between January 5 and 10, 1939, blood was transferred into 4 lambs from sick sheep, from 50 to 700 cc. into each in a single session. These lambs were killed after 6 months, none of them displaying any signs of jaagsiekte.

### WORMS AND JAAGSIEKTE

The causative relationship between lung worms and jaagsiekte has been discussed by M'Fadyean<sup>10</sup> and by me,<sup>1,9</sup> both reaching the same conclusion, *viz.*, that lung worms are not directly responsible for the

disease. One of my experiments, in which a lamb was brought up under worm-free conditions and yet contracted the disease, apparently through air-borne infection, points to a direct transmission without any interaction of worms. Serial sections of primary nodules have in some cases revealed *Muellerius* larvae, but in others, none.

On the other hand, when the difficulty of direct transmission was realized, I could not reject the possibility of some intermediary agent. Since every sheep in this country is infected with the lung worm, *Muellerius capillaris*, and this worm is as strictly limited to sheep as is jaagsiekte, no other animal being susceptible to *Muellerius*, we thought that this parasite might possibly be an intermediary host to the virus, possibly in a similar way to that which Shope<sup>11</sup> has found for lung worms of hogs and rain worms which harbor the virus of swine influenza, and Syverton and Berry<sup>12</sup> for the woodtick as transmitter of equine encephalomyelitis. The following experiments were made with this possibility in mind.

The first experiment was made with larvae from jaagsiekte lungs, in order to see whether they might harbor the virus. When that experiment was negative, I thought that the lung worms might "prepare the soil," eventually weaken the tissue, and lower its resistance against the attack of the virus. With the possibility in mind that the sexual hormones of the worms might enter into this combination, perhaps by stimulating epithelial growth, I thought that the alveolar linings might be less resistant in the immediate vicinity of developing worms and therefore more susceptible to contact with the virus when the worms were reaching full development.

The larvae of *Muellerius capillaris*, which are passed by the bronchial secretion into the mouth and from there to the intestinal canal, are found in the feces in variable numbers according to how massive the lung infestation is, but with marked periodic variations as systematic countings have shown. These larvae are eaten by snails, especially land molluscs (*Agriolimax agrestis* and *arion*). I have frequently found the larvae in *Agriolimax*, of which young specimens are found in abundance in the pastures. I fed young snails (*Agriolimax*) with *Muellerius* larvae and kept the snails for 2 to 4 weeks to allow the larvae to develop. Then the snails were fed to lambs, 4 to 8 to each lamb at a time, and this procedure was repeated several times for 2 weeks with each lamb.

#### *Larvae from Jaagsiekte Lungs*

In August, 1939, 6 lambs were fed repeatedly with worm-infested snails. A culture of pasteurilla-like bacilli of pneumonia<sup>13,14</sup> was repeatedly injected intratracheally during the experimental period. None

of the lambs showed any clinical symptoms of jaagsiekte. Six months later all 6 lambs were killed. No lung changes were found except in one a milium-sized nodule which histologically resembled jaagsiekte, but the picture was not typical.

### *Larvae from Jaagsiekte-Free Lungs + Filtrate*

In parallel with the above experiment, starting at the same time, August, 1939, larvae from jaagsiekte-free lungs were obtained and fed to snails and then to 8 lambs. On November 17, 1939, 3 months after the snails were ingested, a mixture of 4 parts Chamberland L2-filtrate of fresh jaagsiekte tissue and 1 part *Pasteurella pneumonia* broth-culture, was injected intratracheally, 5 cc. into each lamb. Eight months after the experiment was started all 8 lambs were killed. In 3, typical jaagsiekte lesions were found in the lungs.

It may here be observed that inoculations with the *Pasteurella pneumonia* organisms were performed repeatedly by various routes, but this organism was never found to produce epithelial proliferations nor anything resembling jaagsiekte when injected alone. Yet its frequent association with the later stages of the disease led me to make numerous experiments with this microorganism, injecting it intratracheally and intrapulmonally into 21 lambs in all and keeping those which did not succumb from the initial pneumonia for 4 to 8 months, with the possibility in mind that the adenomatous proliferation might be a post-infectious process. No trace of epithelial proliferation was found.

### IS THE VIRUS INTRAEPITHELIAL?

Intentionally, the findings in direct smears have not been mentioned before in this report. Frequently some bacteria are found, sometimes in great numbers and particularly in cases associated with acute inflammation of the lungs.

In a flock where jaagsiekte is prevalent, some sheep may be attacked suddenly by acute pneumonia, and I usually find then the *Pasteurella*-like bacillus of contagious pneumonia,<sup>13,14</sup> but in other cases hemolytic streptococci may be found. As already mentioned, there is reason to assume that these organisms have no relation to jaagsiekte and must be regarded as concomitants.

One of the most regular findings in smears is to see the protoplasm of a great number of mononuclear cells more or less crowded with vacuoles of a fairly uniform size. These cells are mostly desquamated cells from the alveolar epithelium, but partly also columnar cells from the bronchial tree and the adenomatous tissue. With higher magnification, the protoplasm of these cells is seen to contain minute corpuscles, surrounded by a clear zone. Sometimes a large part of the



protoplasm appears to be filled with these minute corpuscles, as if a great number of them were contained in one large vacuole. The corpuscles themselves are so small that they are just visible at the highest magnification (Fig. 13). No characteristic inclusion bodies have been found. The corpuscles mentioned have been stained with Giemsa's, Paschen's and Castaneda's stains. They are just as distinctly brought out in photomicrographs as by direct examination of stained smears.

One has the impression that these minute corpuscles cause an accumulation of fluid in their immediate surroundings, and that an accumulation of them in a cell will cause an increase in the water content of the protoplasm. If this supposition is right, it might account for the greatly increased water-content in the lungs in these cases and the increased bronchial secretion might be a reaction to the irritative stimulation of the virus particles.

#### COMMENT

The cause and nature of jaagsiekte have been problematical, and de Kock,<sup>6</sup> who is one of the most experienced authors on this subject, is inclined to classify this disease among the tumors. The histological picture certainly makes that conclusion probable, and the difficulty encountered by all experimenters might point in the same direction. The problem, however, is not whether the disease should be classified in one category or another but crystallizes around the fundamental question of etiology: What is the cause of jaagsiekte and how is the disease transmitted? I do not pretend to have found a satisfactory answer to this question. Certain facts appear, however, to have been established:

Jaagsiekte is an infectious disease, which is transmitted by the exhaled respiratory air. It is probably caused by a virus, which grows intracellularly in the alveolar and bronchial epithelium. The possibility that the virus may penetrate the placental circulation cannot be rejected, for, although I have never seen the disease in newborn lambs, there are descriptions by reliable persons, from which one can infer that the disease has been observed in lambs 2 weeks old. This, however, seems to occur very rarely. I have never seen the initial stage of the disease in lambs younger than 4 months, which would indicate a postnatal infection. The difficulty in transmitting the disease with unfiltered lung material may be caused by a virus-neutralizing agent in the transferred cells.

Some of my experiments seem to indicate that the disease can be transferred through exhaled air alone. Yet we cannot estimate the possible rôle of lung worms, which are present in every sheep lung in this

country. My experiments indicate that lung worms (*Muellerius capillaris*) are not vectors for the virus. Yet their presence and activity, perhaps in a certain stage of development, may enhance the action of the virus and facilitate its initial development in the alveolar tissue.

Likewise, the bacillus of contagious pneumonia may be of importance in lowering the resistance of the tissue. Although not alone able to cause the disease, it is easier to produce jaagsiekte with a filtrate if it is injected with a culture of this bacillus.

In spite of the difficulty in transmitting the disease, its infectious nature seems to be beyond doubt.

Since no bacteria have been found with any constancy, the assumption of a virus as the infectious agent seems to be the only possible conclusion. The positive results with filtrates tend to confirm that hypothesis. Although cultivation in developing eggs has been unsuccessful (and I have transferred chorionic material from the first inoculated eggs through 8 generations without visible results), that does not exclude the possibility of a virus since it may be, and probably is, one of slow development, as indicated by the long period of incubation.

I am inclined to think that the mononuclear exudative cells are derived from the alveolar lining and that these lining cells may proliferate without losing contact with the alveolar wall, to form an epithelial tuft which may be the beginning of an epithelial growth. Under certain circumstances the nuclei of these cells seem to divide without a corresponding division of their protoplasm, and so giant cells with many nuclei may be formed (Fig. 14). In this respect jaagsiekte would bear a certain resemblance to giant cell pneumonia, as it has been described in children by Hecht,<sup>15</sup> Moore and Gross,<sup>16</sup> Masson and Paré,<sup>17</sup> Karsner and Meyers,<sup>18</sup> and recently by Pinkerton, Smiley, and Anderson.<sup>19</sup> The virus of jaagsiekte might be of plasmodium-like nature, as suggested by Masson and Paré for the giant cell pneumonias, and the minute corpuscles seen in the cells of jaagsiekte lungs somewhat resemble Histoplasma, although of much smaller size. Yet, in contrast with giant cell pneumonias, inclusion bodies are not conspicuous in jaagsiekte.

As already mentioned, I have never seen cases resembling jaagsiekte in man, although we have been looking for it in our autopsy material during the last 8 years. The cases published by Bonne,<sup>20</sup> Bell,<sup>21</sup> and Ikeda<sup>22</sup> of adenomatosis in human lungs resemble jaagsiekte more or less histologically, but since no such cases have been found here, not even among the shepherds, who are in daily contact with sick sheep in closed houses for long periods of time and should have every opportunity of contracting the disease, we must conclude that man is im-

mune to this particular virus. The growth stimulation of this virus is no unique phenomenon, for several irritating substances can cause epithelial proliferation, although only exceptionally to such an extent as the virus of jaagsiekte.

### CONCLUSIONS

Experimental production of jaagsiekte is bound with great difficulties. There is every reason to conclude that the infectious agent is contained in the expiratory air, and my experiments indicate that the sheep are infected by inhaling the exhaled breath from sick sheep. Experience from this and other countries corroborates this conclusion.

On the other hand, it seems to be practically impossible to convey the infection by means of the watery discharge from the bronchi, which one would expect to contain the virus in great amounts. I have taken this secretion directly from sick sheep and have injected it immediately, warm and unfiltered, into healthy lambs, intrapulmonally and intratracheally, but without result.

Also it seems to be practically impossible to reproduce the disease by injecting unfiltered ground tissue from typical jaagsiekte lungs. Filtered material gave a higher percentage of positive results, but yet the rate of takes was so low that this must be termed a very unreliable method of inoculation. When employed in combination with a broth culture of the bacillus of contagious pneumonia and/or developing lung worms there seemed to be a greater chance of positive results with filtrates. And as both these agents are present in practically every flock and *Muellerius capillaris* is present in every sheep in this country, they might at least in part be responsible for the unusually severe spread of the disease here, although the main cause of the high morbidity rate must be considered to be the housing conditions, for the sheep are kept closely housed for weeks and months under conditions which are ideal for spreading a respiratory infection.

Whether the minute corpuscles reproduced in Figures 13 and 14 are the causal organisms of jaagsiekte I am unable to prove at present. But since these corpuscles are found with greater regularity than anything else resembling organisms I venture to reproduce these photomicrographs as suggestions to investigators.

### SUMMARY

Various attempts have been made to reproduce jaagsiekte by inoculating filtered and unfiltered material from affected lungs. Only very few of these gave positive results, particularly with unfiltered material.

Infection was readily brought about by exposing healthy lambs to exhaled air from sick sheep.

Positive results were also obtained by making sick sheep breathe through glycerine-saline solution and then injecting the fluid, unfiltered and filtered, into lambs intratracheally.

Transmission by filtered extracts of tissue proved easier in combination with intratracheal injections of bacillary cultures causing pneumonia in sheep and particularly in sheep which had been fed with snails infested with lung worms of sheep. Yet the lung worm (*Muel-lerius capillaris*) seems not to be a vector of the virus.

Jaagsiekte is concluded to be due to a pneumotropic virus, strictly limited to the lungs and bronchi of sheep and excreted with the respiratory air.

Photomicrographs are presented of intracellular corpuscles which are tentatively supposed to be virus corpuscles.

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#### DESCRIPTION OF PLATES

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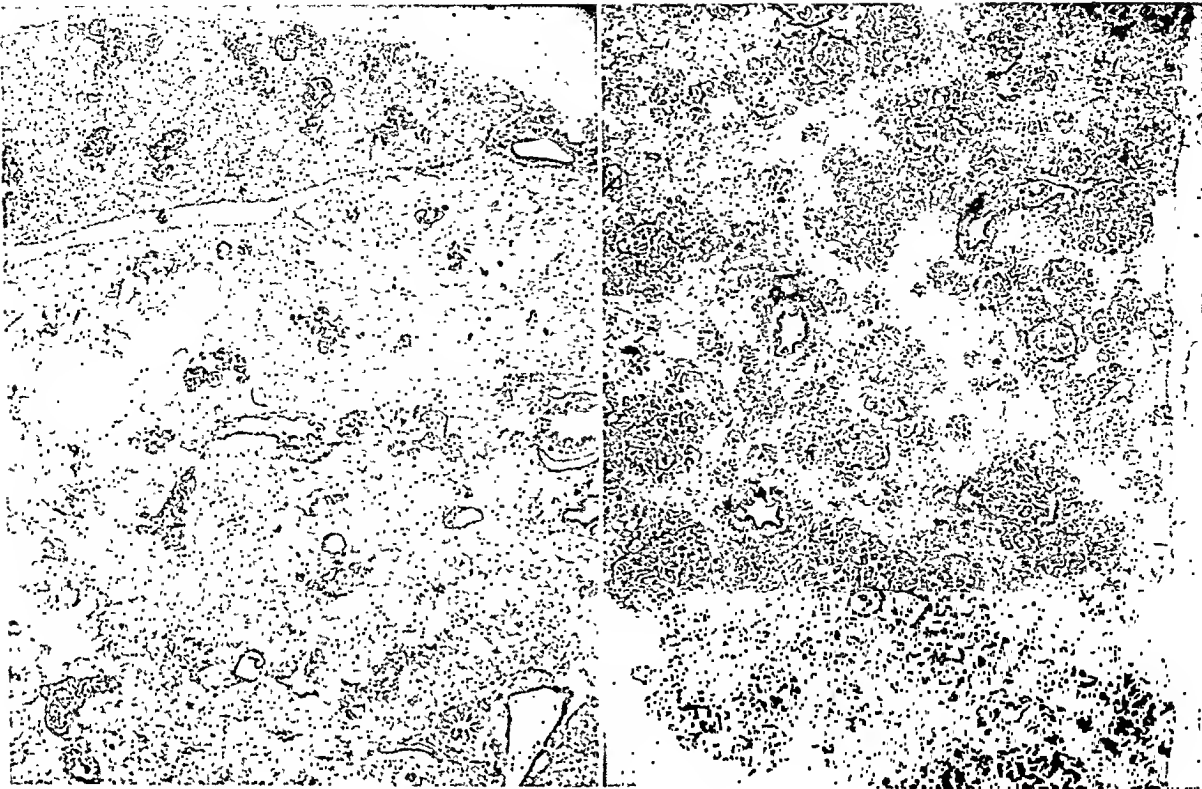
##### PLATE 143

FIG. 1. Initial lesions of jaagsiekte.  $\times 10$ .

FIG. 2. Initial lesions with pronounced intrabronchial proliferations.  $\times 10$ .

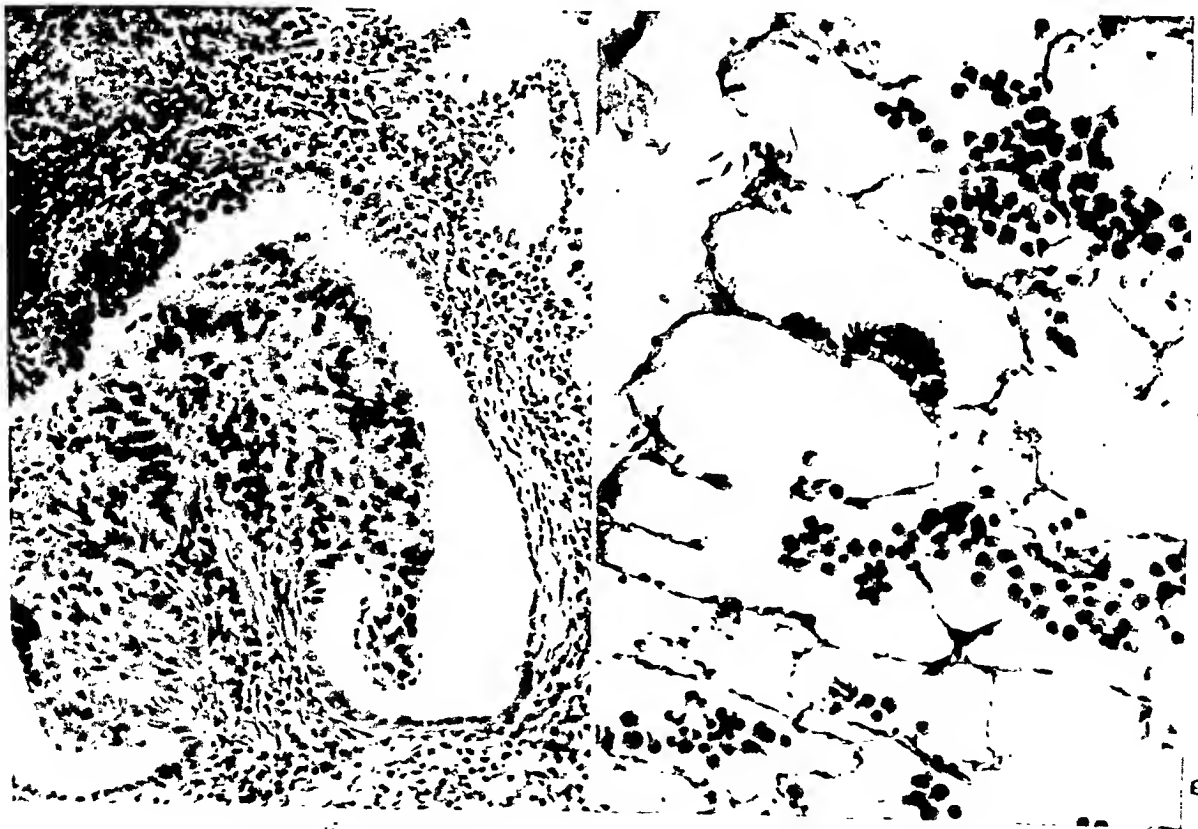
FIG. 3. Inflammation and epithelial proliferation in bronchiole.  $\times 280$ .

FIG. 4. Epithelial tuft originating from alveolar lining. Mononuclear cells in alveoli.  $\times 280$ .



1

2



3

4

Dungal

Experiments with Jaagsiekte

PLATE 144

FIG. 5. Lymphoid hyperplasia surrounding adenomatous proliferation in bronchioles.  $\times 70$ .

FIG. 6. Intrabronchial adenomatous proliferation.  $\times 260$ .

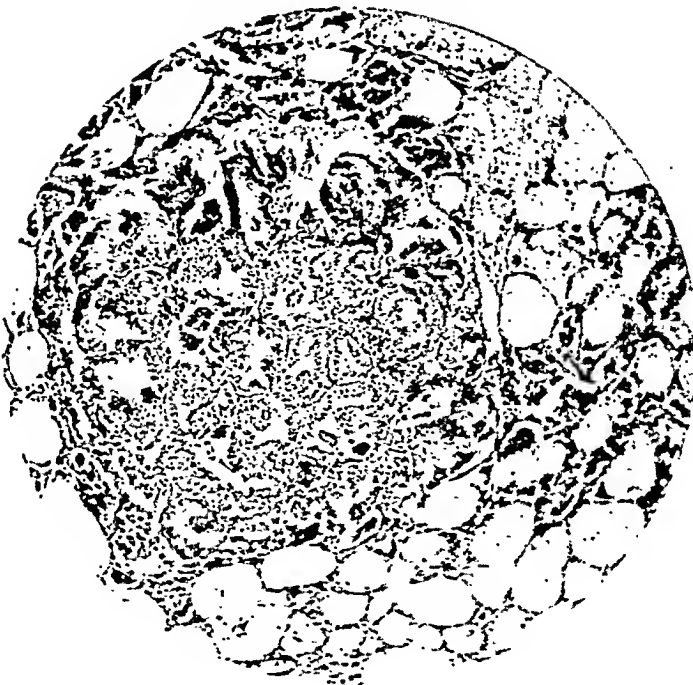
FIG. 7. Isolated adenomatous nodule.  $\times 70$ .



5



6



7

Dungal

Experiments with Jaagsiekte

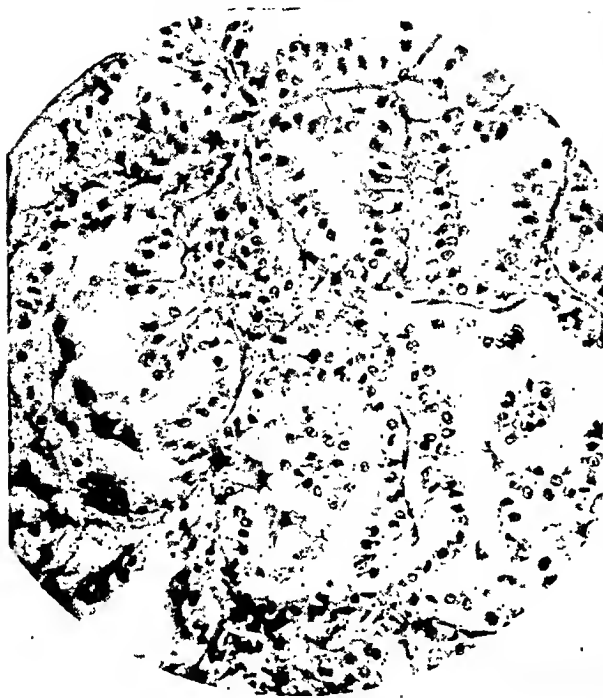


PLATE 145

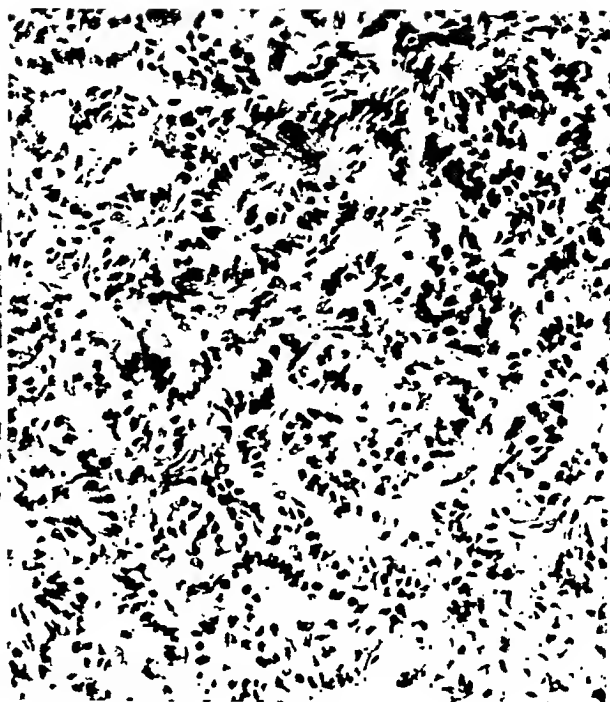
FIG. 8. Regular columnar cells with clear protoplasm, mounted on delicate fibrous strands.  $\times 240$ .

FIG. 9. Irregular growth, resembling carcinoma.  $\times 280$ .

FIG. 10. Advanced case of Icelandic jaagsiekte. A normal lung shown on the right, for comparison.



8



9



10

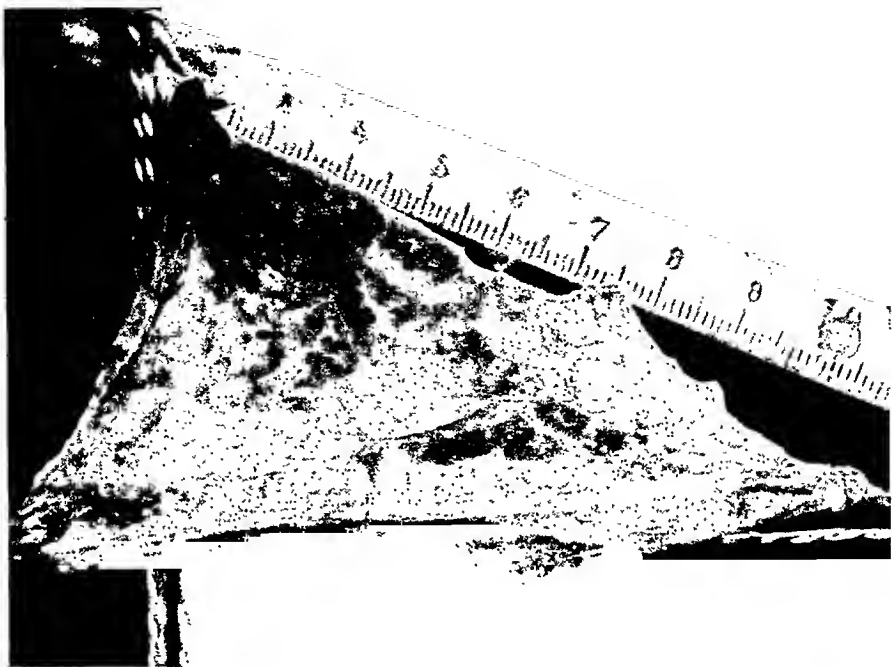
Dungal

Experiments with Jaagsiekte

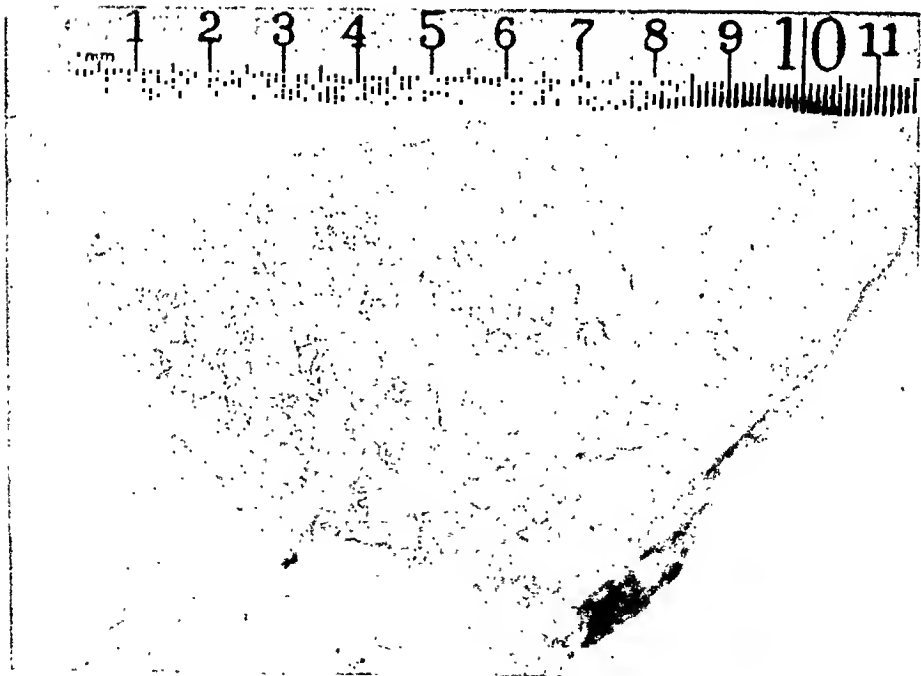
PLATE 146

FIG. 11. Mottled surface of adenomatous lung.

FIG. 12. Cut surface of adenomatous lung tissue.



11



12

PLATE 147

FIG. 13. Intraepithelial virus? Minute corpuscles, each surrounded by a clear zone, in protoplasm of epithelial cell. Giemsa's stain.  $\times 1600$ .

FIG. 14. Giant cell in section from jaagsiekte lung. Of note are the minute Plasmodium-like corpuscles in protoplasm.  $\times 1600$ .

FIG. 15. Experimentally produced jaagsiekte, 4 months after injection of 5 cc. of Berkefeld-N filtrate intrapulmonally.  $\times 28$ .



13



14



15

Dungal

Experiments with Jaagsiekte



CHRONIC LEPTOMENINGITIS AND EPENDYMITIS CAUSED BY  
USTILAGO, PROBABLY U. ZEAE (CORN SMUT)

USTILAGOMYCOSIS, THE SECOND REPORTED INSTANCE OF  
HUMAN INFECTION \*

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It is well known that certain fungi which are saprophytic in their more common or natural habitat are pathogenic for man, and at times produce significant disease. The following saprophytic fungi are proved pathogens for man: *Coccidioides immitis* (coccidioidomycosis) may be found in the air or in the soil; *Candida* (*Monilia*) *albicans* (moniliasis) may be a saprophyte in nature; species of *Actinomyces* or *Nocardia* (actinomycosis) can be isolated from the soil; *Zymonema* (*Blastomyces*) *dermatitidis* (North American blastomycosis) is in all probability a saprophyte; *Paracoccidioides brasiliensis* and *P. cerebriiformis* (South American blastomycosis or Lutz-Splendore-de Almeida disease) can be found as saprophytes on coffee plants; *Phialophora verrucosa* and other organisms which cause chromomycosis (chromoblastomycosis) can be found growing saprophytically on logs or decaying wood; *Sporotrichum schenckii* (*sporotrichosis*) has been found on barberry bushes and other plants as a saprophyte; *Aspergillus*, *Mucor*, and other fungi which produce human diseases are air-borne; *Rhinosporidium seeberi* (rhinosporidiosis) in all probability is water-borne. There are, no doubt, other pathogenic fungi producing human disease that have saprophytic existences, the sources of which are unknown.

It has been frequently suggested, but seldom proved, that fungi pathogenic to plants might, under favorable conditions, parasitize man. It is a well known fact that certain mycotic phytopathogens can act as antigens to produce sensitization in man. Cadham,<sup>1</sup> in 1924, had three patients who developed asthma from contact with grain rusts. The chief fungus thought to be responsible for the disease was the wheat rust, *Puccinia graminis*. Hopkins, Benham, and Kesten<sup>2</sup> reported a case of asthma in which the inciting cause was an *Alternaria*. Several species of *Alternaria* produce diseases in plants. Among these may be listed *A. mali* (apple leafspot disease), *A. solani* (early blight of potatoes), and *A. panax* (ginseng blight). Other investigators found, as did Brown,<sup>3</sup> that patients were sensitive to numerous fungi that in certain instances were plant pathogens.

\* Received for publication, July 23, 1945.



Actual invasion of human tissues by plant pathogenic fungi, however, is rare, in spite of the large number of fungi saprophytic on plants which may affect man. Of particular interest is the case reported by Preininger<sup>4</sup> of a 31-year-old farmer who spent the night in a corn field in a drizzling rain after working in the field under a scorching sun. An examination of the patient revealed that he had a symmetrical arrangement of skin lesions which corresponded to the areas in contact with the wet clothing. These consisted of infiltrated, hyperemic patches on the chest, back, arms, inguinal region, and legs, with scaly plaques in the axillae, on the neck, elbows, and dorsum of the feet. There were scattered red papules on the chest, legs, and buttocks. The hyperkeratotic epidermis of the palms and soles was raised in the form of large sheets of lamellae and revealed an infiltrated corium. A microscopic examination of scrapings from the lesions disclosed spores which were similar to those seen in the black, smutty areas on corn leaves brought from the field in which the patient had slept. The cutaneous disease was thus identified as being caused by the corn smut, *Ustilago zeae* (*U. maydis*).

The above-described case is of interest for two reasons. The first is that it conclusively proves that fungi pathogenic for plants can cause human disease and, secondly, because we have been unable to find in the literature any other case of human infection with *Ustilago*. It is probably the first proved instance of human infection by *U. zeae*.

Because of the rarity of human disease caused by *U. zeae*, for which the term ustilagomycosis is suggested, it is thought that the following case of chronic leptomeningitis and ependymitis caused by *Ustilago* is worthy of recording. It is, to the best of our knowledge, the second instance of human infection with this organism and the first in which the infection has involved an internal organ, the brain.

#### REPORT OF CASE

*Clinical History.* The patient was a white married man, 55 years old, who was admitted to Barnes Hospital on April 29, 1942, and died on May 7, 1942. He complained of a swimming sensation in his head and a staggering gait that had become worse during the 4 months prior to his admission.

The past history revealed that in 1937 he had had an attack of nausea and vomiting for which an operation was performed and a midline epigastric incision was made, but nothing was found. In 1938 he was admitted to a hospital in Louisville, Kentucky, because of staggering, nausea, slight headaches, and attacks of vomiting of 2 years' duration. A cerebellar craniotomy was performed, but no tumor was found and a diagnosis of "chronic cystic arachnoiditis" was made without biopsy. The craniotomy wound did not heal normally and there was continuous drainage from the wound for approximately 4 months. The patient had had defective hearing for a long period. There was no history of meningitis.

Following the cerebellar craniotomy the patient felt well and returned to work as

a farmer which included, among his chores, that of husking corn. He continued to feel well until 4 months before entering Barnes Hospital.

Physical examination revealed a somewhat gruff, almost deaf farmer. At times he seemed unable to understand well, but otherwise he was clear, oriented, cooperative and did not appear to be acutely ill. There was a right lateral nystagmus. Hearing was definitely impaired, more so in the left ear. Since his first illness he had had trouble with his memory and there had been blurring of vision. Adiadokokinesis was present in the left arm. The Romberg test revealed a tendency to fall forward and to the left. The ocular fundi were normal. The reflexes were normal except the abdominal reflexes which were absent. The pulse rate on admission was 56 beats per minute, and the blood pressure was 125/70 mm. Hg. Subsequently the pulse rate increased to 72 beats per minute. On the day before death the pulse varied between 100 and 126, and finally rose to 130 shortly before death. The blood pressure was 125/70 mm. of Hg on admission, reached 138/88, and was 86/74 shortly before death. The temperature fluctuated between 37° and 38° C. and terminally rose to 39° C.

The routine laboratory studies were not remarkable.

On May 5, 1942, a ventriculogram was made, air being injected through a previous perforator opening after 110 cc. of clear fluid were removed from the ventricle. Plates showed the air only in one ventricle. Air was then injected into the left ventricle, following the removal of 60 cc. of fluid. The plates revealed a moderate, symmetrical dilatation of the lateral ventricles, of the third and fourth ventricles, and a prominent aqueduct of Sylvius. Because the ventriculographic studies showed only moderate dilatation of the ventricular system and there were no signs of pressure, it was thought that surgical intervention was not indicated. Thirty-six hours after these studies, the patient developed irregular breathing, became cyanotic and unconscious, and died. Terminally, there were clinical signs of bronchopneumonia that did not respond to chemotherapy and oxygen.

The final clinical diagnoses were hydrocephalus, probably of degenerative type, and bronchopneumonia.

#### NECROPSY

The external examination of the body disclosed the healed surgical wound in the posterior occipital region, but otherwise nothing remarkable was noted.

The weight of the lungs was moderately increased, the combined weight being 1250 gm. There were scattered fibrous adhesions over the surfaces of both lungs. Irregularly outlined foci of gray, brown and red consolidation, varying from a few mm. to 2 cm. in diameter, were found in all lobes of the lungs. The intervening parenchyma was subcrepitant and gray to pinkish red. A small amount of frothy mucus was present in the trachea and bronchi. A calcified nodule, 2 mm. in diameter, was present in the pulp of the spleen. There were small yellow foci in the intima of the pulmonary, cerebral, and coronary arteries. Similar foci were present beneath the endocardium of the anterior leaflet of the mitral valve. In the wall of the aorta and the splenic arteries there were raised yellow plaques. Except for small deposits of fat beneath the endocardium of the left ventricle, there was nothing remarkable in the heart. There were no gross pathologic changes in the liver, spleen, skin, kidney, or gastrointestinal tract.

*Examination of the Brain.* The occipital bone showed the trephine openings and the occipital craniotomy. The dura mater was unusually tense and the cerebral convolutions were flattened. A pointed glass tube was inserted into the right lateral ventricle and clear fluid was obtained under pressure. There were fibrous adhesions between the cerebellum and the dura mater. There was a herniation of the cerebellar tonsils into the foramen magnum. The leptomeninges over the cerebral hemispheres and over the base of the brain were slightly thickened and light grayish white. No pathologic change was noted in the basilar arteries.

Following fixation in 3 per cent neutral formaldehyde, the brain was sectioned in a coronal plane, the sections being taken at distances of from 1 to 4 cm. apart. There was moderate symmetrical dilation of the lateral, third, and fourth ventricles. The aqueduct of Sylvius was widely patent, measuring 4 mm. in diameter. The foramina of Luschka and Magendie were obliterated by fibrous adhesions in the subarachnoidal space. There was a faintly visible fine granularity of the ventricular ependyma. There was no gross pathologic change in the brain tissue.

#### *Microscopic Examination*

*Lung.* Several sections taken from the foci of consolidation showed essentially the same pathologic change. The alveoli and bronchi contained polymorphonuclear leukocytes, fibrin, macrophages, and scattered red blood cells. The alveoli in the remaining parenchyma were partially filled with a lightly eosinophilic-staining precipitate which appeared finely granular.

*Brain.* Sections from the cerebral cortex, cerebellum, and pons (stained with hematoxylin and eosin) showed a chronic inflammatory change in the leptomeninges. Moderate numbers of lymphocytes, plasma cells, and macrophages were present, with a moderate increase in connective tissue. Occasionally a grouping of a multinucleated giant cell and macrophages suggested a tubercle. In the perivascular spaces of Virchow-Robin, adjacent to the pia-glial membrane, there were lymphocytes and plasma cells, in certain instances filling the space.

The section through the medulla including the fourth ventricle disclosed a broad zone of chronic granulation tissue replacing the normal ependymal surface (Fig. 1). In the granulation tissue were large numbers of lymphocytes, plasma cells, large mononuclear macrophages, epithelioid cells, and multinucleated giant cells of the Langhans' type. There were scattered eosinophils and polymorphonuclear leukocytes. The giant cells demonstrated a remarkable variation in size and shape. The peripherally arranged nuclei were usually vesicular and the cytoplasm in most instances was homogeneous, but occasionally contained

fine vacuoles. The chronic inflammatory tissue lining the ventricle contained branching, yeast-like forms of a fungus within many of the giant cells, occasionally in macrophages, and scattered among the inflammatory cells in the granulation tissue. In the sections stained with hematoxylin and eosin the fungi were seen in faint outline, giving the cell a vacuolated appearance, suggestive of phagocytized fat (Fig. 5). In sections stained by the Gram-Weigert method, the fungi were intensely colored and were seen clearly as hyphae or chains of budding or sprouting mycelium. Some of the fungi showed branching (Figs. 2, 3, 13, and 14) and others presented a whorling configuration (Fig. 4). Clear zones surrounded the fungus within the giant cells in practically all instances. These zones were interpreted as the result of a lytic action on the part of the growing fungus.

A post-mortem culture of blood from the heart on blood agar revealed only diphtheroids, regarded as contaminants.

### Anatomic Diagnoses

Chronic mycotic leptomeningitis and ependymitis; moderate internal hydrocephalus; healed wound of an occipital craniotomy; cerebellar pressure cone; bronchopneumonia of all lobes of the lungs; lipoidosis of the pulmonary, cerebral, and coronary arteries; arteriosclerosis of the aortic and splenic arteries; and fatty infiltration of the myocardium.

### MYCOLOGY

The classification of a pathogenic fungus is usually determined by studying the evolution of the organism in artificial mediums and by identifying the various morphologic and physiologic entities which comprise the genus and species. For one trained in mycology, it is usually not difficult to identify, at least generically, most fungi in tissue. An exact identification of the fungus causing the intracranial disease in this case was difficult chiefly because the organism could not be studied on artificial mediums. A chronic infection, especially one due to a fungus, was not suspected at the time of necropsy to account for the slight opacity and thickening of the leptomeninges. Consequently, the entire brain was fixed in formaldehyde, making cultures impossible. Moreover, the appearance of the cells in the tissue, when first examined, gave no clue to their identity. The examination of serial sections of the block of tissue taken from the fourth ventricle, where the organisms were found, revealed that these mycotic structures were apparently morphologic forms known as sprout mycelium. Sprout mycelium is a form of vegetative mycelial growth which develops under certain conditions of nutrition and environment.

A review of the known human mycoses failed to reveal sprout my-

celium in parasitized human tissue of the type observed in this case. The fungi of such granulomatous diseases as blastomycosis, paracoccidioidal granuloma, and histoplasmosis are seen in tissue chiefly as budding cells, either simple or multiple. The organisms causing chromomycosis are seen as simple or multiple cells with septum or cross-wall formation, but no buds, while the characteristic structure of the organism of sporotrichosis is a cigar- or oval-shaped cell. The fungus of coccidioidomycosis or the progressive form of the disease, coccidioidal granuloma, is seen as an endosporulating structure. The fungi causing actinomycosis and maduromycosis when observed in tissue are in the form of branching fine filaments, bacillary cells, or granules composed of filaments and spores. *Candida* (*Monilia*) *albicans* and its related organisms, the cause of moniliasis, a disease which may have a systemic distribution but is usually cutaneous, shows budding, yeast-like cells in tissue. Under certain conditions, *C. albicans* may show filaments or pseudomycelium, but never of the type seen in the case described here. The dermatophytes, including such fungi as produce microsporosis, trichophytosis, epidermophytosis, endodermophytosis or tinea imbricata and favus, present filaments, spores, or chains of cells which may be branching, but are not sprout cells of the type found in this case. Furthermore, the dermatophytes cannot be considered as systemic invaders in the sense that they produce internal lesions of a serious nature, particularly of the brain. Likewise, there is a marked difference in appearance of the hyphae and spores of *Aspergillus*, *Mucor*, *Penicillium*, and other genera of the so-called "weeds of mycology" which may produce systemic disease.

A clue to the probable identity of the fungus was found only after studying the organism in all its forms in the serial sections. This clue was a germinating spore which was engulfed by a giant cell (Fig. 6). The outer wall of the spore appeared thick and had surface markings which consisted of small excrescences or spines. The germinating portion of the structure appeared to be protruding through a split wall, was thin-walled, nonstaining or hyaline in character, and blunt at the growing tip. A combination of the sprout mycelium plus the spiny, germinating cell suggested the characteristics of a smut organism. This supposition was further strengthened by the finding of a spore in a clear space within a macrophage which was characteristic of an immature or young smut spore with the thick, dark wall and the single nucleus (Fig. 7). Further search in other slides from the fourth ventricle and also in the choroid plexus of the fourth ventricle was rewarded by the finding of an elongated, irregularly elliptical, spiny or echinulate spore (Fig. 8), a chain of echinulate spores (Fig. 10), and

masses of spiny spores and germinating forms in the choroid plexus (Figs. 11 and 12). For the most part, the spiny spores were globose (spherical) with the exception of a few which were subglobose to ellipsoid or irregular. The mature globose spores that were observed measured approximately 7 to 10  $\mu$  in diameter, whereas the elongated spores were approximately 11  $\mu$  in the long axis.

The Ustilaginales or smuts include several hundred species which parasitize higher plants.<sup>5</sup> Of this large number, many can be eliminated in an attempt to classify the fungus in the case presented here on the basis of spore size and spore surface markings or absence of markings. The cereal smuts are the more common forms encountered on farms. The work of Stakman<sup>6</sup> has been of great help in eliminating certain cereal smuts on the basis of spores and spore germination.

*Ustilago hordei*, the covered smut of barley, can be eliminated since the mature spores are smooth and do not have spines. *Tilletia foetens*, the stinking smut of wheat, likewise has smooth-walled spores. In *Ustilago tritici*, the loose smut of wheat, the spores are lighter on one side than on the other and the spines are usually seen on the lighter side, sometimes along the edge on optical section and sometimes covering only half the spore. The spore wall is rarely split by the promycelium and usually the promycelium is constricted at the base, precisely where it emerges from the spore. Since the spores observed in our sections showed spines over the entire wall, splitting of the spore wall and no constriction of the germination at the base, it is safe to eliminate this organism. *U. nuda*, the cause of loose smut of barley, has spores which resemble those of *U. tritici*. The spores of this organism appear hollowed out in the center, giving them a concave appearance. This feature was not observed in the spores seen by us. The spores of *U. avenae*, the smut of oats, resemble those of *U. tritici* and *U. nuda*. The spores of *U. zae*, also known as *U. maydis*, the cause of corn smut, have characteristics similar to those of the organism noted in the sections from our case.

In plants, *U. zae* produces what is known as a smut tumor. The tumor is covered with a thin membrane which encloses the mass of powdery spores in addition to the parenchymal cells and fibrovascular bundles of the host. When the membrane ruptures, the mature spores are set free and scattered by the wind or drop down to contaminate the soil in the vicinity. The mature spores measure approximately 7 to 12  $\mu$  in diameter, are usually spherical, but may be ellipsoid or irregular in shape and vary less from the normal than do the spores of *U. tritici* and *U. nuda*. The spores are brown, and are not lighter on one side than on the other, but the individual spores may vary some-

what in density, some being darker around the edges, especially on optical section, appearing dark brown to black. The outer wall or *epispore* is covered with prominent spines or warty excrescences which show distinctly on all sides. Under satisfactory conditions of temperature and moisture the spores will germinate, but when unfavorable they will remain dormant, retaining their viability for several years or until such time as growth conditions are again favorable.

The relation of temperature to germination was studied by Jones<sup>7</sup> who found the optimum temperature for corn smut to be between 26 and 34° C., the maximum between 36 and 38° C., and the minimum 8° C. The optimum is higher than for most smuts and helps make clear two important points. The first is that this tolerance to high temperature explains why corn smut is more severe in the warmer regions where corn is grown, and the second is that that may be the reason why the fungus is able to produce lesions in the human body.

When the mature spore germinates, it forms a special structure known as the *promycelium* which is made up of four somewhat elongated cells. From these promycelial cells there develop, laterally and terminally, secondary structures termed *sporidia* which are fusiform and vary in size. Under continued favorable conditions, the promycelium will branch and produce a large number of sporidia. Some of the sporidia will become elongate to form so-called infection threads, while others will bud as do yeasts to produce secondary sporidia or sprout cells. When the budding cells reach the air or when there is a changed oxygen tension, the sprout cells form branches or chains of cells, many irregular in shape and size. These break off and are scattered by the wind to germinate and produce localized tumors when they reach young corn plants. The time for the development of mature spore sacs, depending upon the environment, varies from approximately 1 to 3 weeks. More information can be had by reading one of the standard texts on plant diseases such as that of Heald.<sup>8</sup>

It is apparent from the foregoing that the various stages of the fungus that we have observed in human tissue strongly suggest *Ustilago*, probably *U. zae*, as the causative agent of the infection. In summary, these stages consist of: the sprout mycelium which grows under altered conditions of nutrition and oxygen tension; the young or immature spore which shows the characteristic thick wall and nuclear structure; and finally the mature, germinating spores with the varied shapes and sizes, and spines or warty excrescences. Unfortunately, conclusive proof of the identity of the pathogen, namely, its cultivation on artificial mediums, was not possible since the brain was fixed in formaldehyde before it was known that the disease was caused by a

microorganism. However, in spite of the lack of cultural studies we feel justified in considering this case as an example of systemic disease caused by a smut organism that was in all probability that of corn, *U. zeae*.

### DISCUSSION

It is difficult to hazard a reasonable guess as to the portal of entry of the fungus. A review of the case history, however, suggests at least two possibilities. The first possibility is that of contamination through the drainage tract following cerebellar exploration, and the second is by way of the gastrointestinal tract. In favor of the first is that shortly after the operation the patient went to work on the farm, and included among his chores was that of husking corn. Although on first thought this appears as a plausible explanation, it does not explain the genesis of the patient's intracranial disease prior to the operation upon which a diagnosis of chronic cystic arachnoiditis was made. Moreover, the draining wound following craniotomy would suggest that the disease was present before the operation, a reasonable explanation for the failure of the wound to heal normally. In favor of the second possibility is that prior to the cerebellar craniotomy the patient had had an attack of nausea and vomiting of such severity as to lead to a laparotomy. It would seem, therefore, that conclusive evidence is lacking to substantiate either of these possibilities, but that the gastrointestinal tract appears to be the more likely portal.

It has been known for a long time that corn smut contains an active principle, probably an alkaloid, which exerts an action similar to that of ergot. The alkaloid, when ingested by cattle, affects the nervous system to produce what is known as "staggers" (grass staggers or stomach staggers).<sup>9</sup> Highly nitrogenous feeds have also been blamed for causing this disease. In this respect it is worth while to mention that analyses of corn smut have shown that it has a high carbohydrate content and more protein than is found in corn, oats, or clover hay. The symptoms of staggers vary, but generally they indicate involvement of the nervous system. The first symptoms are somnolence and hypoactivity with subsequent general signs of frenzy. The animal is constipated and the output of urine is small. The urine is darker than usual. There may be trembling and spasms of muscles in different parts of the body. In the dull stage, respirations are depressed and each expiration may be accompanied by a sound like snoring. The pulse rate is low, but the volume is good. When aroused suddenly from the drowsy state, the animal appears startled and stares wildly. When moving about it may stagger, with the hind quarters swaying from side to side.



In the advanced stage, when delirium sets in, a cow is said to be mad, having such symptoms as bellowing, stamping of feet, running about wildly, grating of teeth, and frothing at the mouth. There is muscle twitching and jerking and the body may become covered with perspiration. These symptoms are frequently accompanied by convulsions followed by a prolonged period of coma. When consciousness is regained, the animal may get up on its feet, quietly eat some food; or blindly stagger about. Not all of these symptoms, however, are always present in the same animal. When the symptoms of drowsiness are present it is called "sleepy staggers," and when the symptoms are those of frenzy it is called "mad staggers." Frequently the animal will be paralyzed and remain so until death. Post-mortem examination reveals congestion of the brain, meninges, and lungs.

Ergotism is a rare disease in man and is uncommon in America. Ergot-like symptoms caused by *U. zea* (*U. maydis*) and termed ustilaginism by Mayerhofer<sup>10</sup> likewise are rare. Von Storch,<sup>11</sup> in 1938, listed the symptoms and signs of the *gangrenous* and *convulsive* or *neurogenic* types of ergotism in man following the use of ergotamine tartrate in the treatment of migraine headache. As described, the two types when combined bear a striking similarity in many respects to the combined symptoms of ustilaginism described by Mayerhofer<sup>12</sup> in children.

According to von Storch,<sup>11</sup> the symptoms and signs of 42 cases of ergotism showed with the gangrenous type lassitude, dullness, vague lumbar pains, cramps in the calves of the legs, and dull burning pain in the extremities followed by waves of heat or cold leading to numbness. There were also noted vomiting, and swelling of the feet with the skin of the extremities becoming cold and reddish violet, and developing vesicles which preceded the blackening of the gangrene which was usually dry. Jaundice was commonly observed. The convulsive or neurogenic type was characterized by fatigue, heaviness of head and limbs, giddiness, insomnia, excitement leading to delirium and mania, with impaired sight or hearing and formication. There were painful spasms of the face, throat, and diaphragm; tonus, clonus, myoclonus, contractures, myopia, myosis, vomiting, diarrhea and amenorrhea. Pseudotabetic, hemiplegic, or paraplegic symptoms have been described.

The symptoms observed in a number of cases of ustilaginism by Mayerhofer<sup>10, 12</sup> are remarkably similar to the symptoms of ergotism described by von Storch.<sup>11</sup> Mayerhofer described intense itching of the body and the extremities; itching, redness, and swelling of the nasal and buccal mucous membranes; marked scaling of thick, dry

crusts on the soles and palms; either intense sweating or none, with usually an elevated temperature in the beginning; muscle weakness and rheumatic types of pains; redness, swelling, and heat in the distal part of the extremities followed by pigmentation of the affected parts; gastrointestinal disturbances following the ingestion of corn flour containing corn smut or ergot; diminishing desire to eat and strong feeling of thirst; gastro-enteritis and colic; gangrene of the extremities, usually dry; latent or manifest tendency toward spasms, eclampsia-like symptoms, cramps, contractures, catatonia, delirium, mania; increased blood pressure with tachycardia preceded in the beginning by bradycardia; exacerbation of symptoms by sunlight; rapid cure if diagnosed early; severe disease in the late stages, with possible death.

It is easily seen from the foregoing that ergotism and ustilaginism in man and animals have enough symptoms and signs in common so that they may be regarded as similar toxic phenomena. In addition to the clinical aspects, post-mortem findings in cases of ergotism in man usually reveal hyperemia, edema, and hemorrhage in the gastrointestinal tract, lungs, and brain. These observations, although not diagnostic of the disease, are generally those seen in animals dying from eating corn smut.

It is interesting to note that there were observed in the patient studied here symptoms similar in many respects to those described for "staggers" in animals. The patient's chief complaint was staggering; he had had gastrointestinal disturbances of a proportion to necessitate an abdominal operation. Other complaints suggesting ergotism included nausea, vomiting, and some neurologic signs. The general clinical picture, however, was not that observed in the most advanced cases of ergotism or ustilaginism. Gangrene of the extremities, marked scaling of the skin, redness and swelling of the mucous membranes, and the more advanced neurologic signs were not observed in this patient. It should be noted, however, that the severest symptoms and the most advanced lesions were not observed in all cases of ergotism and ustilaginism.

The pathologic changes produced by the ingestion of the toxic principle of *Ustilago* in animals are most marked in the brain (leptomeningitis) with an accompanying edema and congestion of the lungs. In rats, Tichomirov and Bogdanovic<sup>13</sup> observed fatty degeneration of the myocardium with some fragmentation of the fibers, degenerative changes in the kidneys with foci of calcification, and thickening of the intimal layer of blood vessels with hyalinization of the cells. In human ergotism the changes noted are hyperemia, edema and hemorrhage in the brain, lungs, and gastrointestinal tract.

The pathologic changes observed in the case reported here are compatible with the aforementioned changes observed in spontaneous and experimental *Ustilago* poisoning in animals. However, it should be emphasized that the observed pathologic changes described for *Ustilago* intoxication are not in any way to be regarded as in themselves sufficiently characteristic to be diagnostic of this particular intoxication. In the patient studied here there were observed pathologic changes in the leptomeninges and the ependyma which were the site of an inflammatory disease produced by the microorganism. At best, congestion and edema of the brain are difficult to evaluate properly when the examiner is looking for them. These changes were not looked for specifically at the necropsy.

It is interesting to note that there was a deposition of lipid material in the intima of several arteries and beneath the endocardium of the left ventricle. Comparable changes have been described by Tichomirov and Bogdanovic<sup>13</sup> for their rats with experimentally induced ustilaginitis. However, it is impossible to attach great significance to these pathologic changes since they are so frequently seen in routine autopsies.

#### SUMMARY

A case of chronic leptomeningitis and ependymitis caused by a species of the genus *Ustilago*, *U. zeae*, the corn smut fungus, is described. No other focus of infection was found in the body and it was not possible to determine with certainty the portal of entry and how the leptomeninges and ependyma became infected. The microorganism was identified by the finding of characteristic germinating spores in the fourth ventricle. Histologically, the fungus produced a granulomatous reaction in the leptomeninges and the ependyma with giant cells of the Langhans' type within which the fungus frequently produced sprout mycelium. Clinically, the patient manifested some of the signs and symptoms that have been described in man and animals affected with ergotism and ustilaginitis.

This case is believed to be the first instance of systemic infection produced by *Ustilago* to be reported, and the second proved case of human infection with an organism of this genus. The term, ustilagomycosis, is suggested for the granulomatous tissue change produced by fungi of the genus *Ustilago*.

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[ Illustrations follow ]

## DESCRIPTION OF PLATES

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### PLATE 148

All photomicrographs were made from sections of the posterior portion of the fourth ventricle.

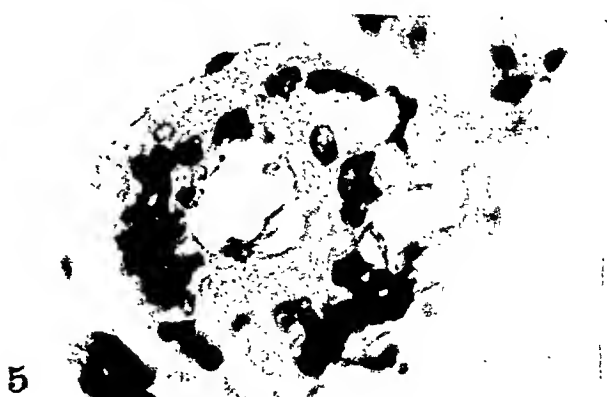
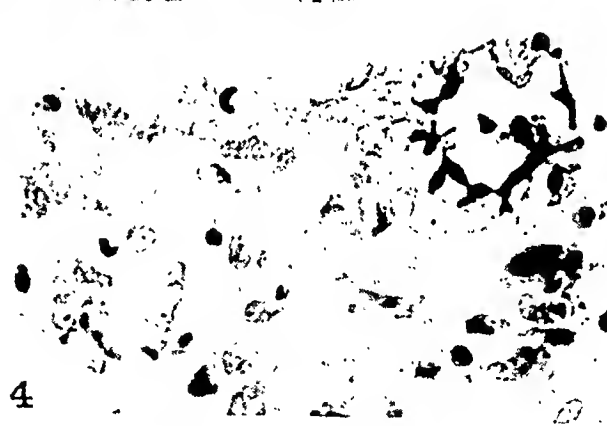
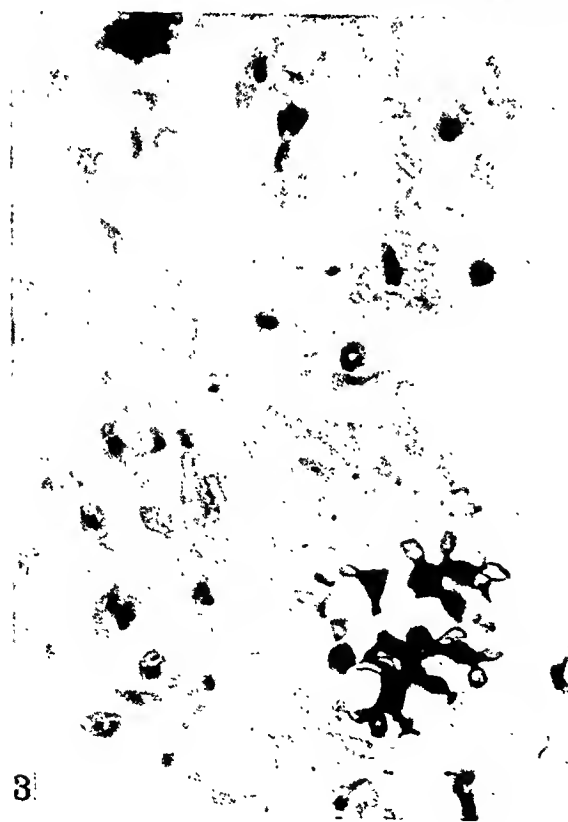
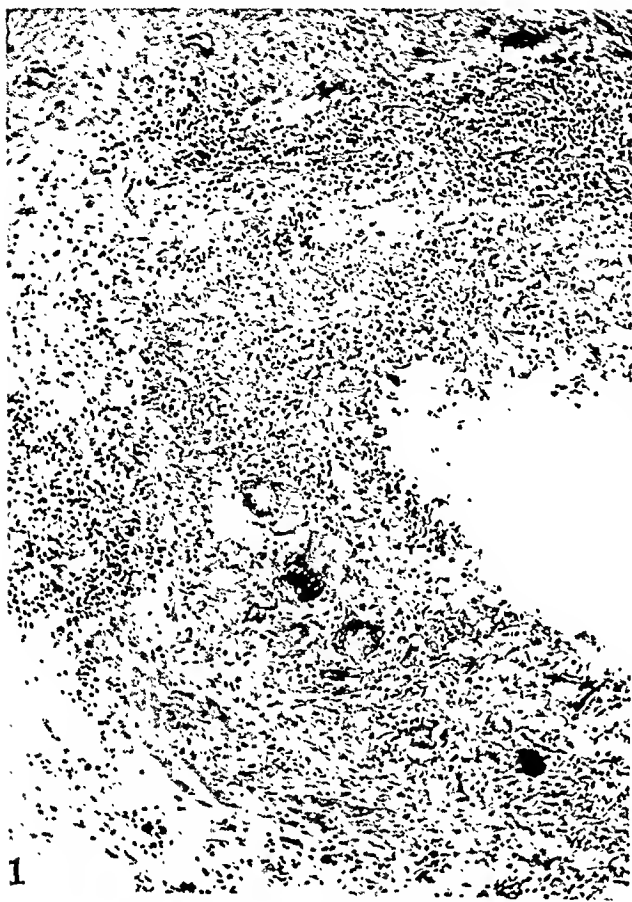
FIG. 1. Granulomatous reaction in tissue, showing giant cells and cellular infiltrate. Gram-Weigert stain.  $\times 95$ .

FIG. 2. Giant cells in meningeal tissue showing sprout mycelium. Gram-Weigert stain.  $\times 480$ .

FIG. 3. Giant cells engulfing organisms. Gram-Weigert stain.  $\times 650$ .

FIG. 4. Giant cells engulfing organisms. Gram-Weigert stain.  $\times 495$ .

FIG. 5. Giant cell showing clear zones within which are faintly stained fungus cells. Hematoxylin and eosin stain.  $\times 530$ .



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PLATE 149

All photomicrographs were made from sections of the posterior portion of the fourth ventricle.

FIG. 6. Germinating, mature spore of *Ustilago* in giant cell. Of note are the thick, spiny episore and faint, blunt beginning of the promycelium. Gram-Weigert stain.  $\times 1510$ .

FIG. 7. Young, immature spore in macrophage. Of note are the thick wall and clear zone around the fungus cell. Gram-Weigert stain.  $\times 1510$ .

FIG. 8. Elongated, spiny or echinulate spore. Gram-Weigert stain.  $\times 1510$ .

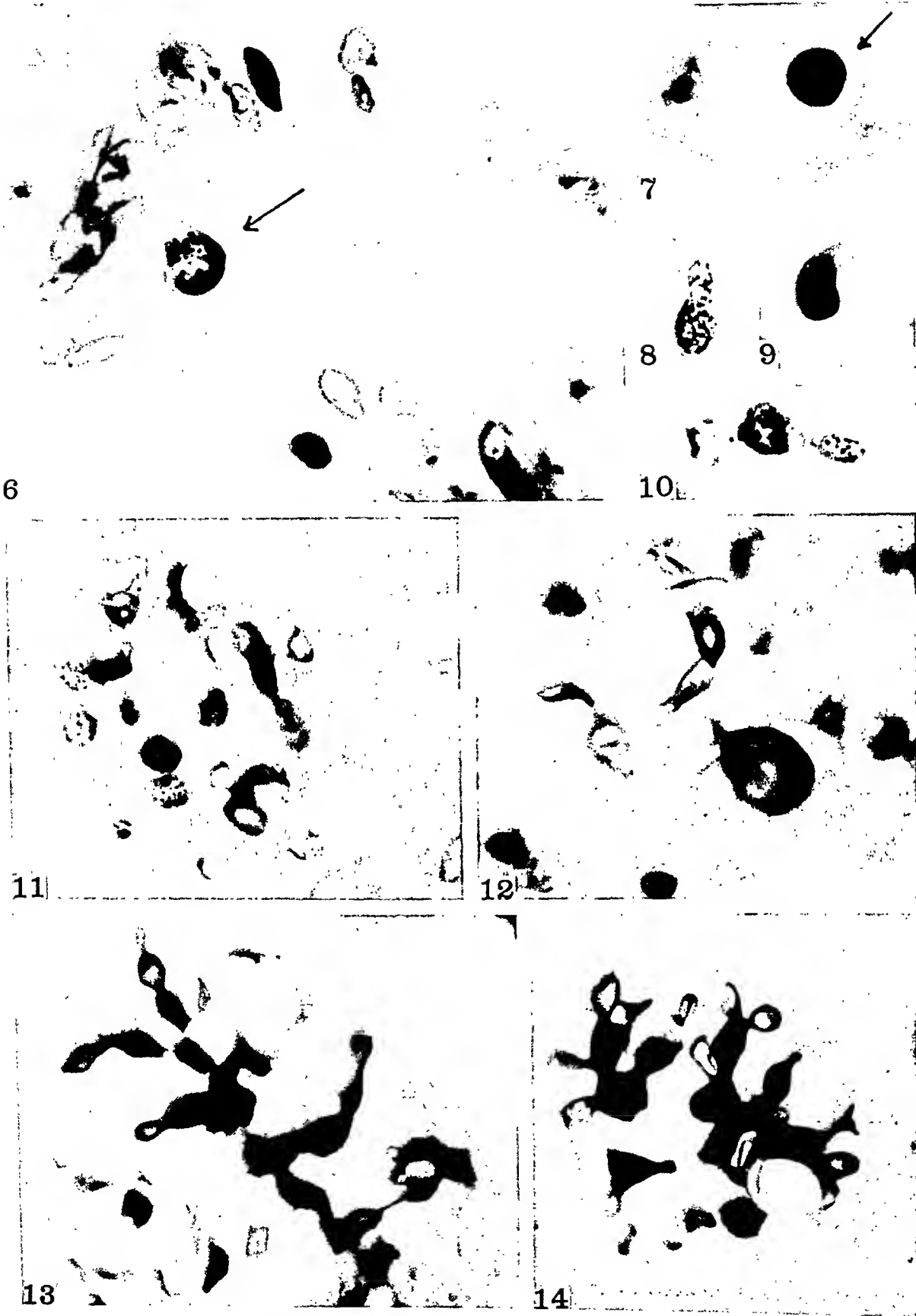
FIG. 9. Germinating cell showing development of promycelium. Gram-Weigert stain.  $\times 1510$ .

FIG. 10. Chain of echinulate spores. Gram-Weigert stain.  $\times 1510$ .

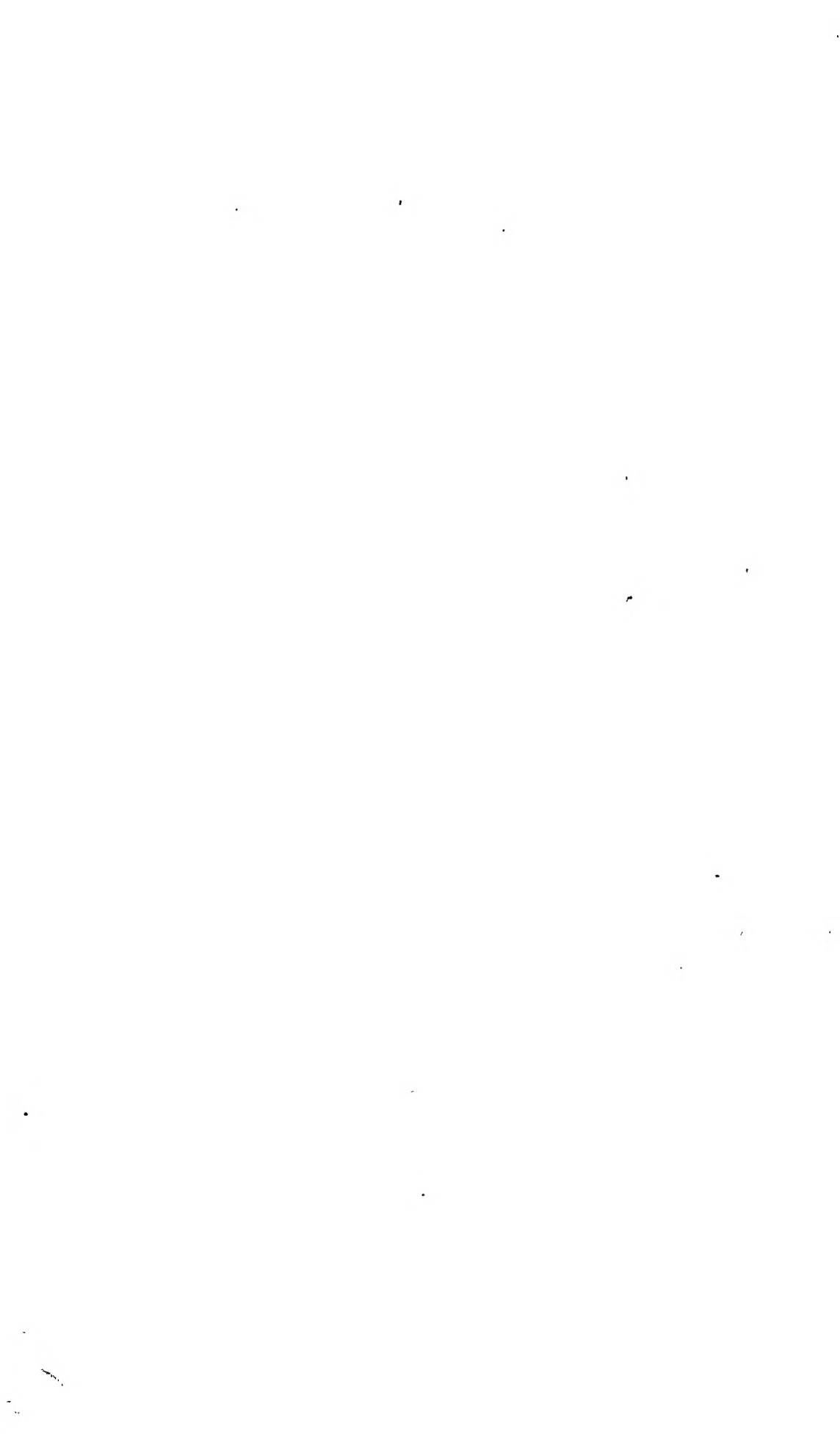
FIGS. 11 and 12. Groups of mature germinating cells in the choroid plexus. Gram-Weigert stain.  $\times 1510$ .

FIG. 13. Sprout mycelium. Gram-Weigert stain.  $\times 1290$ .

FIG. 14. Sprout mycelium, showing budding. Gram-Weigert stain.  $\times 1290$ .







## SYSTEMIC INFANTILE TOXOPLASMOSIS \*

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The number of cases of toxoplasmosis, reported since human infection by this protozoon became unquestionably established, indicates that it is a disease of increasing clinical importance. The world-wide distribution of *Toxoplasma* in animals and the widespread geographic areas from which human cases have been reported show that its epidemiology is potentially of great significance. The variable clinical and subclinical manifestations that result from toxoplasmic infestation and the unsolved problems regarding its transmissions are factors which make the recording of any additional information desirable. We have recently studied a newborn infant who died of a fulminating and widely disseminated toxoplasmic infection which we will describe in detail. This is the first instance of the disease in South Carolina, and, so far as we have been able to determine, is the first case to be reported from the southeastern states. Only 21 cases proved by necropsy are recorded in the literature. Of these, 15 cases, including the one reported here, occurred in infants, 2 in children, and 4 in adults. In 12 of these cases organisms were found in tissues outside the central nervous system (Table 1).

### REPORT OF CASE

A boy, weighing 5 lb. 11½ oz., was born to healthy white parents on December 26, 1943. The parents had been married for 18 months and it was the woman's first pregnancy. Labor and delivery were uneventful. The infant was slightly cyanotic at birth, particularly about the face. The cyanosis became more intense and generalized. Edema developed which pitted on legs and abdomen and was brawny and indurated over thighs, buttocks, back, upper arms, and face. Respirations were normal and the pulse regular. There were no heart murmurs. The liver and spleen were palpable, the former being 3 fingersbreadth below the costal margin. The cyanosis persisted and the abdomen became distended. The infant had no bowel movements and on rectal irrigation mucous casts and a little meconium were obtained. The edema was gradually absorbed, but the baby became steadily weaker. Five days after birth he developed distinct signs of cerebral irritation with nystagmus and recurring attacks in which the eyes rolled upward, accompanied by fixation of muscles of the face and irregular movements of the arms. Convulsive movements became nearly continuous shortly before death on December 31, 1943.

The parents were natives of Hendersonville, N. C. The father was 23 and the mother 21 years of age. They had lived in a trailer camp in the congested area adjacent to the Charleston Navy Yard for 11 months immediately prior to the baby's birth. The only animal with which they had come in contact was a dog. This pet was described as healthy. The parents had always been in good health, having had no serious or unusual illnesses. The mother was in an automobile accident when 5 months' pregnant, following which she experienced a few transient abdominal pains. Kline exclusion test of her blood was negative.

\* Received for publication, July 23, 1945.

## REPORT OF NECROPSY

The body was that of a well developed and apparently full-term male infant whose sclerae and skin were lemon yellow. There was some puffiness of the face and lids and the skin appeared thickened, although there was no pitting edema.

There were 25 cc. of orange-colored fluid in the peritoneal cavity.

The right lung weighed 33 gm. and the left lung, 35 gm. Both were subcrepitant and reddish purple, with scattered areas of rubbery consistency. The right lung was intensely congested and there were small hemorrhages beneath the visceral pleura. Hemorrhages were present also beneath practically the entire parietal pleura on the right.

The heart weighed 19 gm. The myocardium had a mottled reddish yellow color. Beneath the epicardium, particularly in the atrioventricular groove, were multiple hemorrhagic foci.

The deep-chocolate-colored liver weighed 137 gm.

The spleen weighed 27 gm. and its capsule was dull and coated with strands of greenish yellow material. The pulp was firm and deep purple. A nodule of accessory splenic tissue, 6 mm. in diameter, was present.

The adrenals were of normal size and shape. On section there was a faint rim of yellowish tissue about the soft brownish gray centers.

The kidneys were of normal appearance.

The sigmoid colon was moderately dilated and contained a large amount of sticky, gummy meconium.

The brain weighed 334 gm. The meninges were slightly cloudy, particularly along the sulci, and an increased amount of brown-tinged fluid was present in the subarachnoid space. There was an area of hemorrhage beneath the pia arachnoid over the left parietal lobe adjacent to the median longitudinal fissure. Multiple sections revealed numerous irregular, brownish red and yellow areas scattered throughout the cerebral hemispheres, pons, medulla, and cerebellum. These discolored foci were often slightly depressed and moth-eaten in appearance. They were most conspicuous in the subcortical white matter, dentate nuclei, and basal ganglia, particularly the thalami. No gritty particles or other evidence of calcification were found. Many of the vessels were markedly engorged, and some had perivascular hemorrhages, one in the parietal region measuring 4 mm. in diameter.

*Histologic Findings*

*Heart.* There was widespread inflammatory involvement of the entire heart wall with multiple foci of necrosis (Fig. 1). The myocardium and endocardium were most heavily involved, the inner portion of the myocardium slightly more than the remainder. The reacting cells

consisted chiefly of lymphocytes and histiocytes with moderate numbers of polynuclear leukocytes, most of which were eosinophils. The inflammatory cell collections were variable in size and often ill defined, but they tended to be focal. The infiltration often involved the supporting fibrous framework and the walls of arteries and veins. The areas of necrosis were chiefly myocardial, however. Inflammatory cell collections in the subendocardial connective tissues were often associated with roughening and even erosion of the overlying endothelium. Some groups of immature blood cells were present. Accumulations of toxoplasmata were easily found within the muscle cells (Fig. 2). In longitudinal section these aggregates were ovoid or spindle-shaped and in cross section were round. Smaller groups of organisms were also present in the myocardial fibers and were often surrounded by a clear space (Fig. 3). Individual organisms were found in and about some of the inflammatory foci and in areas of early necrosis. The circumscribed aggregates of parasites were invariably free from accompanying cellular reaction. Toxoplasmata were also found in the endothelial cells of small arteries, the cells being so swollen and filled with organisms in a few instances as nearly to occlude the lumen. The individual organisms were rounded or ovoid and contained a chromatin mass which was usually situated eccentrically or at the larger end (Fig. 3). They measured 3 to 5 by 2 to 3  $\mu$  in diameter, individual organisms generally being larger than those within the pseudocytes.

*Lung.* Many of the pulmonary alveoli were collapsed. Their walls were thickened and infiltrated by mononuclear cells and occasional polynuclear leukocytes. Within the alveoli were clumps of macrophages with foamy or granular cytoplasm and groups of swollen, cast-off, alveolar lining cells. Networks of fibrin, occasionally mixed with leukocytes, were also present. There was intense congestion throughout, with areas of hemorrhage into alveoli, subpleural connective tissue, and fibrous septae. Some alveoli contained a few strands of vernix caseosa and degenerated epithelial cells. Scattered groups of immature blood cells, chiefly of the erythrocytic series, were found. Toxoplasmata were found in the lining cells of the alveoli (Fig. 4) and in the endothelium and walls of blood vessels. Some alveolar cells contained as many as fifty parasites, whereas five were the most ever noted in a single endothelial cell.

*Liver.* Moderate cloudy swelling of hepatic cells was present, with small fat vacuoles scattered diffusely through the cytoplasm of many. The cells showed bile staining and the canaliculi were stuffed with bile. The lumina of nearly all ducts were open, but an occasional small duct was filled with bile. No definite necrosis was found. Numerous hemato-

poietic foci were present, normoblasts being particularly conspicuous and immature cells of erythrocytic type generally predominating over those of the leukocytic series. These blood-forming cells were also abundant in the fibrous stroma of the portal areas and about the larger portal veins. Many of the cells were eosinophilic myelocytes, particularly in the latter locations. Toxoplasmata were found in the Kupffer cells and in the endothelium of small vessels. There were no lesions for which *Toxoplasma* definitely could be held accountable.

*Spleen.* The spleen showed marked increase in the cellularity of the pulp due to the number of hematopoietic cells. Deposits of fibrin were present in the red and white pulp and on the capsular surface. Toxoplasmata were occasionally found in the endothelium of blood vessels, but there were no granulomas or areas of necrosis.

*Kidney.* The cytoplasm of the swollen epithelial cells of the convoluted renal tubules was granular and vacuolated. The tubular lumina contained granular orange-brown material and occasionally in the epithelium there were fine yellowish granules. The glomeruli appeared normal. Two adjacent veins in the pelvic area showed early thrombosis. The interstitial tissues contained a few foci of hematopoiesis. Toxoplasmata were found in the endothelium of arterioles, but they were sparse, and no lesions for which they definitely could be held responsible were found.

*Adrenal.* The inner two-thirds of the adrenal cortex showed profound degeneration with marked cellular swelling and vacuolization of the cytoplasm. Rounded areas of coagulation necrosis were present throughout this zone as well as less numerous and smaller areas of leukocytic and mononuclear cellular infiltration, a few of which resembled the granulomas in the brain. Accumulations of toxoplasmata were easily found and individual organisms, often degenerating, were also present, but these were identified with greater difficulty.

*Stomach.* Small focal areas of necrosis were present in the gastric musculature with infiltration of lymphocytes, histiocytes, and eosinophils. Similar reacting cells were scattered irregularly throughout the wall, but were most heavily concentrated about blood vessels. Toxoplasmata were found in groups within the smooth muscle cells and singly in and about the inflammatory foci.

*Intestine.* The intestine showed edema of the submucosa and subserosa with infiltration of eosinophils and mononuclear cells. In one section the inflammatory reaction was particularly intense and neutrophils were the predominant cells. Toxoplasmata were found in the epithelium of the mucosa, but no organisms could be identified in the muscle coats or in the most heavily inflamed portion.

*Pancreas.* Edema of the connective tissue framework was found in the pancreas with diffuse infiltration of eosinophils, lymphocytes, and histocytes, and an active vasculitis. Toxoplasmata were found in the vascular endothelium and acinar epithelium.

*Diaphragm.* Perivascular infiltration of mononuclear cells was present in the diaphragm, with a few small areas of early necrosis in the muscle, some fibers of which contained accumulations of toxoplasmata.

*Bone.* In sections of thoracic vertebrae the cortical bone and trabeculae were of normal appearance. The medullary tissue appeared moderately hyperplastic, many of the cells being erythropoietic. An occasional protozoan body was identified, a few in the sinusoids and one in an endothelial cell. The free organisms were fusiform, pyriform, or crescentic.

*Lymph Nodes.* The mesenteric lymph nodes were surrounded by loose fibro-fatty tissue. They showed little definite follicle formation, and the sinusoids were prominent. In addition to the lymphocytic series, immature cells of the granulocytic series, especially myelocytes, were present. Mitotic figures were moderately numerous. Pyriform and ovoid protozoa were found in lymphatic sinusoids, in the swollen endothelial lining cells, and a few were related to blood vessels in the surrounding fibro-fatty stroma.

*Thymus.* Hassell's corpuscles were the site of cellular fragmentation and hyalinization. Eosinophilic myelocytes were present in the medullary tissue. The capillaries were engorged and a single toxoplasma was noted in a capillary endothelial cell.

*Brain.* Numerous small granulomas were scattered throughout the brain, including medulla and cervical cord (Fig. 5). These were composed of collections of epithelioid cells that were often fused into groups in which the cell boundaries were indistinct. The granulomas were closely related to capillaries in nearly every instance, the epithelioid cells apparently being derived from the endothelium of these vessels. Mixed with the epithelioid cells were varying numbers of lymphocytes, polynuclear leukocytes, and eosinophils. Definite necrosis was not a conspicuous feature, but occasionally the reacting cells were arranged about a necrotic center so as to resemble a tubercle. In addition to the granulomas there were areas of edema and degeneration containing small numbers of lymphocytes, large mononuclear cells, and occasional polymorphonuclear leukocytes. In and about such areas the vessels were markedly engorged and there were occasional perivascular hemorrhages. A few vessels contained early thrombi. The meninges were edematous and infiltrated by large numbers of macrophages and large and small lymphocytes. In some places extravasa-

TABLE I  
Cases of *Toxoplasmosis Autenticated by Necropsy*

Author	Location	Sex	Age at onset	Age at death	Clinical features	Tissues involved
1. Janku 18, 10 (Levaditi suggested <i>Toxoplasma</i> )	Prague, Czechoslovakia	M	Birth (?) 3 mos. (?)	11-16 mos. (?)	Hydrocephalus, blindness, convulsions, vomiting, chorioretinitis	Eye; no others examined
2. Richter <sup>5</sup> ( <i>Toxoplasma</i> discovered by Wolf and Cowen)	Chicago, Illinois	F	6 wks.	7 wks.	Convulsions, fever, "cold," opisthotonus	Brain, spinal cord
3. de Lange <sup>9</sup> , 20 ( <i>Toxoplasma</i> found by Wolf and Cowen)	Amsterdam, Holland	F	Birth (?)	4 mos.	Hydrocephalus, labile temperature, tremors of arm, vomiting	Brain
4. Wolf and Cowen <sup>21</sup> (Case 1)	New York City	F	2 days	29 yrs.	Convulsions, labile temperature, slight hydrocephalus, vomiting, diarrhea, respiratory disturbances, chorioretinitis	Brain, spinal cord, eye
5. Wolf, Cowen, and Paige <sup>4</sup> (Case 2)	New York City	M	3 days	31 days	Convulsions, vomiting, signs of cervical cord lesions with sub-arachnoid block, labile temperature, respiratory disturbances, chorioretinitis; enophthalmos	Brain, spinal cord, eye
6. Paige, Cowen, and Wolf <sup>22</sup> (Case 3)	New York City	F	Birth	9 wks.	Hydrocephalus, twitching of lower extremities, labile temperature, convulsions, membranous masses in vitreous, microphthalmos and enophthalmos	Brain, spinal cord, eye
7. Steiner and Kaump <sup>10</sup> (Case referred by Dr. Sailer)	Cincinnati, Ohio	M	Birth (?)	3 days	Hydrocephalus	Brain
8. Zuelzer <sup>11</sup>	Detroit, Michigan	M	Birth (?) 3 days (?)	1 mo.	Listlessness, icterus, cyanosis, rash, labile temperature, labored respirations	Brain, spinal cord, eye

Author	Location	Sex	Age at onset	Age at death	Clinical features	Tissues involved
9. Steiner and Kaump <sup>10</sup>	Detroit, Michigan	M	Birth(?) 1 day(?)	3 days	Petechiae, icterus (erythroblastosis fetalis); fever, cyanosis, twitching of eyes and extremities terminally	Brain; toxoplasmic cysts in adrenal without lesions
10. Torres <sup>9, 10, 23</sup>	Rio de Janeiro Brazil	F	Birth	2 days	Convulsions	Brain, spinal cord, myocardium, skeletal muscle, subcutaneous tissue
11. Hertig <sup>7, 15</sup> (Pinkerton and Weinman identified organism as Toxoplasma; reported by author as sarcocystis)	Boston, Massachusetts	F	?	25 days	2 months premature; fever, diarrhoea	Brain, lung, myocardium, adrenal; very little inflammatory reaction about parasites; died of bacterial infection
12. Paige, Cowen, and Wolf <sup>22</sup> (Case 4)	New York City	M Negro	?	Stillborn	Fetal hydrocephalus, microphthalmos and enophthalmos, unilateral	Brain, spinal cord, eye, myocardium, striated muscle, adrenal
13. Paige, Cowen, and Wolf <sup>22</sup> (Case 5)	New York City	F		3½ days	Respiratory difficulty, edema, cyanosis, mild fever	Brain, spinal cord, lungs, myocardium, adrenals, and ovaries; eyes not examined; toxoplasma, without lesions, found in thyroid, striated muscle and adipose tissue
14. Zuelzer <sup>11</sup>	Detroit, Michigan	M	3 days(?)	11 days	Apathy, drowsiness, cyanosis, convulsions, opisthotonos	Brain, spinal cord, myocardium, lungs, adrenals, kidneys, testicles, striated muscle



TABLE I (Continued)  
Cases of *Toxoplasmosis Authenticated by Necropsy*

Author	Location	Sex	Age at onset	Age at death	Clinical features	Tissues involved
15. Pratt-Thomas and Cannon	Charleston, South Carolina	M	Birth(?)	5 days	Cyanosis, edema, convulsions	Brain, spinal cord, myocardium, lungs, adrenals, stomach, striated muscle and pancreas; parasites without lesions found in liver, bone marrow, spleen, kidneys, lymph node, thymus, and intestine
16. Sabin <sup>13</sup>	Cincinnati, Ohio	M	6 yrs.	1 mo. later	Headache, convulsions, vomiting, weakness of extremities, palpable spleen, fever in latter part of illness	Brain
17. Pinkerton and Henderson <sup>14</sup>	St. Louis, Missouri	M	50 yrs.	8-10 days after onset	Fever, maculopapular rash, diarrhea, pneumonia, oliguria, coma	Lungs, heart and spleen; lesions in skin, but no parasites; brain not examined
18. Pinkerton and Henderson <sup>14</sup>	St. Louis, Missouri	F	43 yrs.	28 days after onset	Weakness, malaise, fever, maculopapular skin rash, chill, headache, pneumonia	Heart, lungs, liver, spleen and brain
19. Pinkerton and Weinman <sup>15</sup>	Lima, Peru	M	22 yrs.	12 days after onset	Weakness, pallor, fever; complicated by <i>Bartonella bacilliformis</i> infection that was apparently subsiding	Brain, heart, lungs, liver, spleen, kidneys, lymph nodes, adrenals, skin, bone marrow
20. Guimarães <sup>16</sup>	Brazil	M Negro	18 yrs.	37 days after onset	Paralysis of lower extremities, nuchal rigidity, dysphasia, fever	Brain, spinal cord, pericardium, kidneys
21. Tomlinson <sup>12</sup>	Canal Zone	F Negress	?	10 yrs. 8 mos.	Died of sickle cell anemia	Toxoplasmic pseudocysts in brain and heart; no inflammatory response

tions of erythrocytes were noted also. The vascular channels in the choroid plexus were intensely engorged, and the stroma of the tufts showed variable degrees of infiltration by large mononuclear cells and lymphocytes. Toxoplasmic pseudo-cysts were easily found (Fig. 6). They were never situated in a granuloma, area of degeneration, or inflammatory infiltrate, although some were fairly close to granulomas. Individual organisms were present in and about the granulomas as well as in the epithelium of the choroid plexus. No calcification was present.

### DISCUSSION

Since the genus *Toxoplasma* was first described in 1908, reports of disease produced by it in animals and man have been rather numerous. Wenyon<sup>1</sup> analyzed several debatable cases reported in human beings and concluded that none of them were due to *Toxoplasma*. He also reviewed much of the literature concerning its occurrence in various forms of animal life in virtually all parts of the world. Olafson and Monlux<sup>2</sup> have recently implicated *Toxoplasma* as the cause of fatal disease in dogs and also have described cases in cats and sheep, thus suggesting a possible source of human infection. Perrin, Brigham, and Pickens<sup>3</sup> have demonstrated toxoplasmic infestation in 8.7 per cent of a group of wild rats studied in the southeastern part of the United States.

Accepted human cases of toxoplasmosis are not of equally wide geographic distribution, for reference to Table 1 shows that 15 cases are from this country. The first case proved by animal inoculation was reported by Wolf, Cowen, and Paige<sup>4</sup> in 1939, following which a review of cases and study of literature by them and others indicated a similar etiologic agent in the cases of Richter,<sup>5</sup> de Lange,<sup>6,20</sup> and Hertig.<sup>7</sup> In addition, they agreed with Levaditi's<sup>8,19</sup> acceptance of *Toxoplasma* as the causative factor in the cases of Janku<sup>9,18</sup> and Torres.<sup>9,23</sup> Steiner and Kaump<sup>10</sup> reported a case of their own and made reference to an unpublished case referred to them by Dr. Seaton Sailer. Recently, Zuelzer<sup>11</sup> published reports on two cases proved by necropsy, and Tomlinson<sup>12</sup> reported a case in which death was due to sickle cell anemia wherein accumulations of toxoplasmata were found in the brain and myocardium.

Of the group beyond infancy, Sabin,<sup>13</sup> who has contributed greatly to the knowledge of this disease, reported a fatal case of toxoplasmosis in a child. Pinkerton and his co-workers have reported two cases in adults who succumbed to a febrile illness similar to the "spotted fever" group,<sup>14</sup> and a third case from Peru<sup>15</sup> complicated by a *Bartonella* infection. Guimarães<sup>16</sup> reported a fatal case in an adult from Brazil.

The main contributors to knowledge in the field of human toxoplasmosis have been the groups associated with Wolf of New York and Sabin of Cincinnati, the former working primarily in the field of clinical medicine, the latter investigating laboratory methods of diagnosis and treatment. Wolf and his associates have described in detail the chorioretinitis and other ocular manifestations of toxoplasmic infection. They also described the multiple foci of intracerebral calcification demonstrable on roentgenographic examination as well as evidence of increased intracranial pressure, and in cases studied with pneumoencephalography, an internal hydrocephalus. Using these findings in conjunction with laboratory methods of diagnosis, their cases include those finally studied at necropsy, as well as clinically accepted cases in which the patients are still alive.

Sabin and his associates early reported toxoplasmic infestation in animals in this country and noted that growth is possible only in living cells. In discussing the pathogenesis of this disease, Sabin stated that intracerebral inoculation of experimental animals resulted in spread of the organisms in the cerebrospinal fluid with marked periventricular tissue destruction. In several reported cases such localization of the reaction leads one to speculate as to whether the choroid plexus served as the initial site in the brain. Such localization was not evident in our case, all indications being that spread was by the blood stream, but some cysts were not related to, or even close to, blood vessels, thus suggesting possible transmission in migrating leukocytes. The histologic changes in this case indicate that the infection was still in the active invasive stage, the process of dissemination of the organisms still being in progress. No new light is thrown on the manner of infection or the portal of entry. The appearance of the lesions, location of the parasites, and complete absence of calcification in the brain lead us to believe that the infection was of short duration, either taking place, or being activated, only a few days before birth. As a matter of fact it cannot be definitely proved that infection did not occur during delivery or immediately thereafter.

We are in full agreement with Steiner and Kaump<sup>10</sup> in regard to the criteria for diagnosis. While conceding the desirability of extensive laboratory examinations on these cases, it seems plausible that cases exhibiting such remarkably distinctive inflammatory reactions and containing morphologically identical organisms should be classified as toxoplasmosis.

The differential staining qualities of the organism have been studied by Perrin<sup>17</sup> and should prove of value in establishing the diagnosis in questionable cases.

The occurrence of jaundice and extramedullary hematopoiesis in toxoplasmosis has been discussed by Steiner and Kaump<sup>10</sup> and by Zuelzer.<sup>11</sup> The former authors were able to make the diagnosis of erythroblastosis fetalis. The jaundice and degree of extramedullary hematopoiesis were impressive in our case, and from a histologic standpoint there is considerable evidence to support the diagnosis of erythroblastosis. However, stimulation of hematopoiesis in sites other than the bone marrow often occurs in the newborn infant due to a number of causes, including infection. Unfortunately, blood studies were not made during life in this case, and without such findings we hesitate to make an independent diagnosis of erythroblastosis until the general toxic effects of *Toxoplasma* are better understood.

### SUMMARY

The described case of toxoplasmosis occurring in a newborn infant is the first to be reported from the southeastern United States.

Toxoplasmata and accompanying lesions were found in the brain, spinal cord, heart, lungs, adrenals, stomach, pancreas, and diaphragm.

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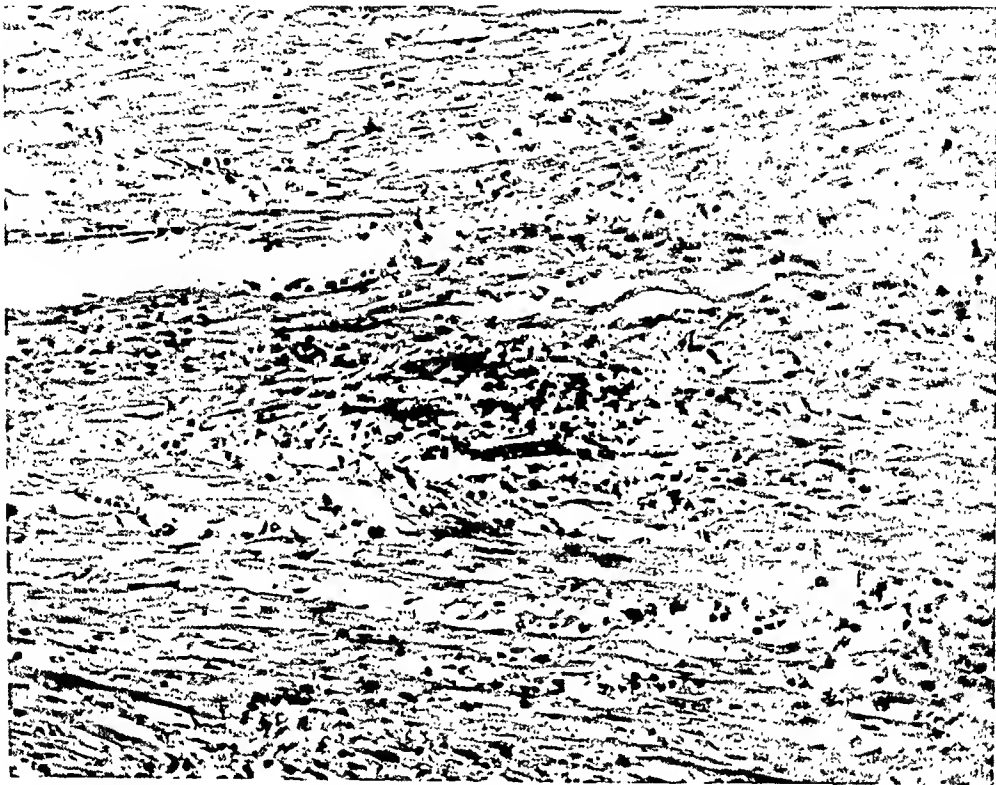
## DESCRIPTION OF PLATES

### PLATE 150

FIG. 1. Heavy inflammatory involvement of myocardium with focal necrosis. Phloxine and methylene blue stain.  $\times 200$ .

FIG. 2. Accumulation of toxoplasmata in myocardium. Hematoxylin and eosin stain.  $\times 1000$ .

1



2



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PLATE 151

FIG. 3. Small group of toxoplasmata in cardiac muscle. Hematoxylin and eosin stain  $\times 1500$ .

FIG. 4. Accumulation of toxoplasmata in alveolar cell of lung. Hematoxylin and eosin stain.  $\times 1000$ .

3



4

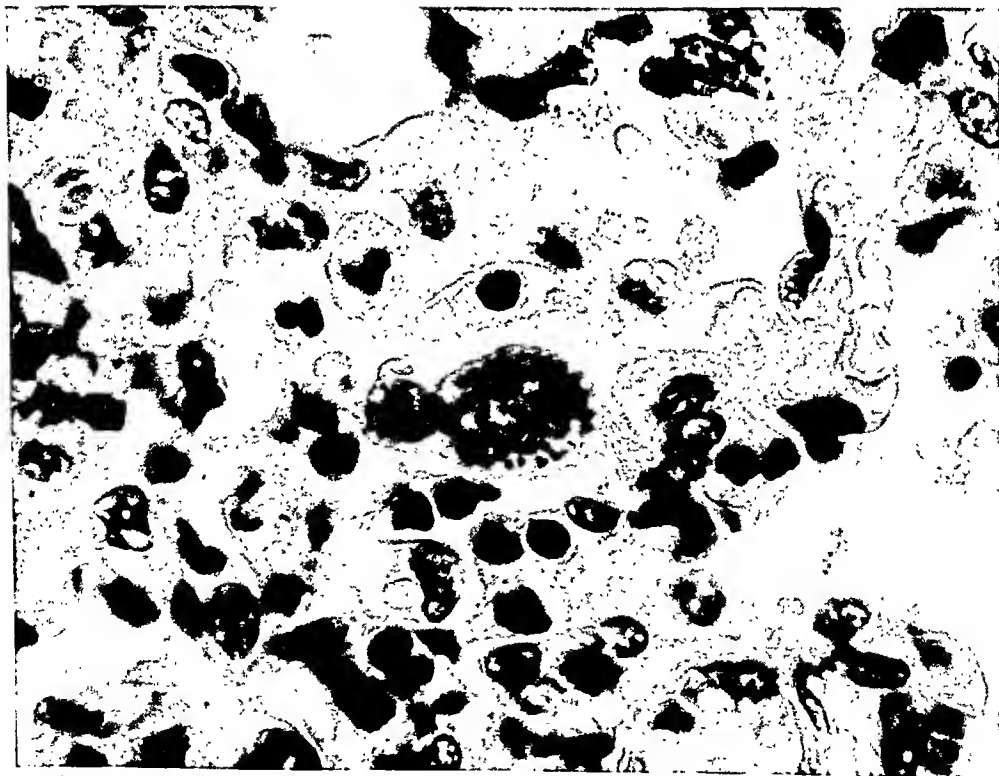


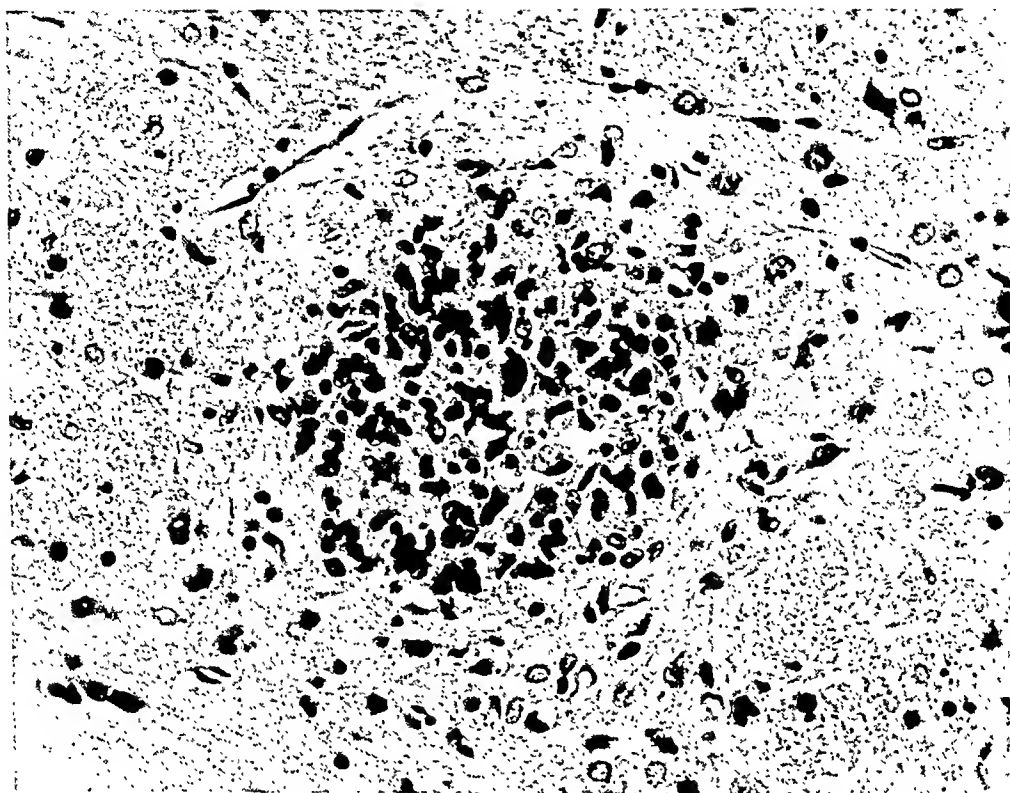


PLATE 152

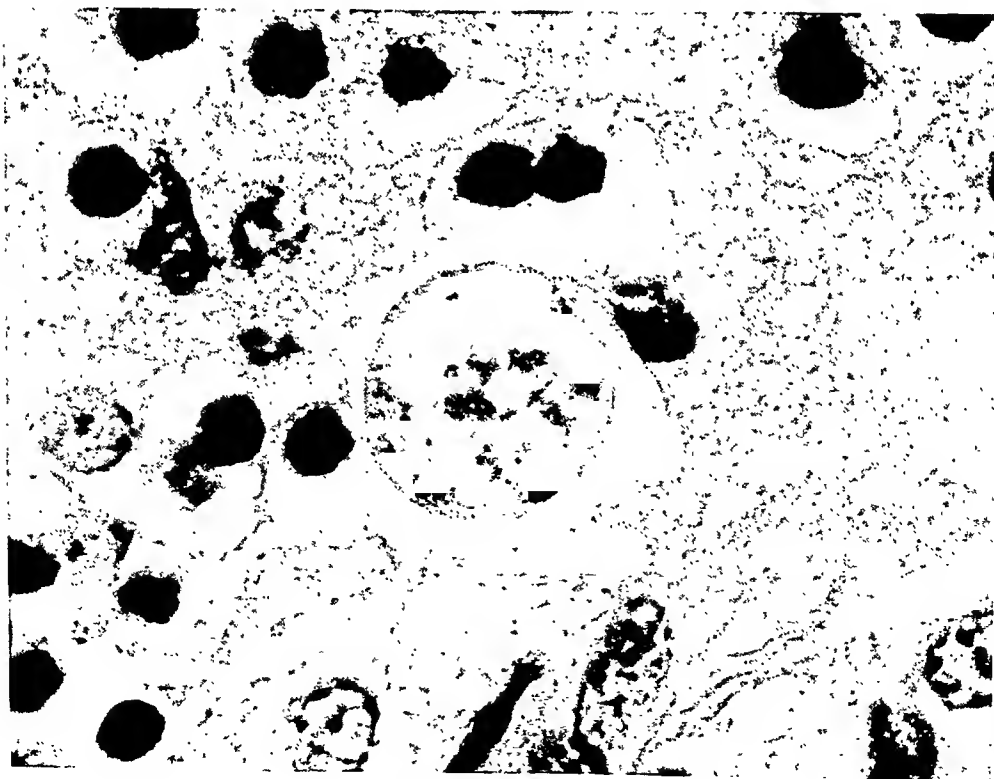
FIG. 5. Typical granuloma in brain. Hematoxylin and eosin stain.  $\times 300$ .

FIG. 6. Toxoplasmic pseudocyst in brain. Hematoxylin and eosin stain.  $\times 1500$ .

5



6



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# PATHOLOGIC FINDINGS IN THE LUNGS OF FIVE CASES FROM WHICH INFLUENZA VIRUS WAS ISOLATED\*.

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Reports in the literature of fatal cases from which influenza virus has been isolated are remarkably few, and still fewer are the descriptions of the pathologic changes in the lungs in such cases. We have been able to find only three such reports. The first case was described by Scadding<sup>1</sup> in 1937, the second by Stokes and Wolman<sup>2</sup> in 1940, and the third by Himmelweit<sup>3</sup> in 1943. The strain of influenza virus was not mentioned in Scadding's case. In Stokes and Wolman's case, it was influenza A (PR 8 strain). Himmelweit's case yielded an influenza virus closely related to, but not identical with, influenza B (Lee strain). All three cases were complicated by *Staphylococcus aureus* infection.

The pathologic change in Scadding's case<sup>1</sup> consisted of a necrotizing process involving the trachea, bronchi, bronchioles, and alveoli. The alveoli were filled with red blood cells, resembling an infarct. In limited areas there were small abscesses, 2 to 3 mm. in diameter.

In Stokes and Wolman's case<sup>2</sup> there was marked congestion of the alveolar capillaries. The alveoli were filled with edema fluid, red blood cells, and a few polymorphonuclear leukocytes. The septa were edematous and the lymphatics were filled with a serofibrinous exudate. Many lobules contained colonies of staphylococci with little or no leukocytic reaction about them. The bronchioles had lost their epithelium and their lumina contained mucus and leukocytic debris. The epithelium of the trachea and large bronchi had desquamated and had been replaced by a thick exudate of organizing fibrin and purulent material. The submucosa was thick, edematous, and congested, and was infiltrated with phagocytes, chiefly of the mononuclear variety.

In Himmelweit's case<sup>3</sup> the lungs showed bronchopneumonia, necrosis near the bronchioles, much hemorrhage, and many staphylococci. The epithelium of the trachea had been shed and there was a little fibrin on its surface with masses of cocci but only a few leukocytes. The bronchial epithelium had likewise desquamated.

In a fourth case reported by Wollenman and Finland<sup>4</sup> from the 1940-41 epidemic, the influenza virus was not isolated but evidence was given for its presence in the lung. A ferret inoculated intramuscu-

\* Received for publication, August 4, 1945.

larly with a suspension of the lung of that case developed no signs of infection, but proved refractory to subsequent inoculation with influenza A (PR 8), and the ferret's serum taken prior to the second inoculation protected mice against infection with this strain of virus. This case, too, was complicated by infection with *Staphylococcus aureus* and the pathologic changes were very similar to those described by Stokes and Wolman.<sup>2</sup>

In addition to these four reports, Andrewes, Smith, and Stuart-Harris<sup>5</sup> recorded the isolation of influenza virus from the lungs of three cases of fulminating pneumonia which occurred during the 1936-37 epidemic in England and from which pure cultures of *Staphylococcus aureus* were obtained. One of these three cases is the same one that was reported by Scadding,<sup>1</sup> but the morbid anatomy of the lungs in the other two cases was not described.

We have had an opportunity to study the lungs from five cases from which an influenza virus was isolated. Two of the cases were unusual in that no significant pathogenic bacteria could be demonstrated, while of the other three, one was complicated by infection with *Staphylococcus aureus*, one by *Staphylococcus aureus* and *Streptococcus hemolyticus*, and one by *Pneumococcus*, type I.

The pertinent gross and microscopic findings, as well as the bacteriologic and viral studies, are given below.

## REPORT OF CASES

### Case 1\*

A white woman, 26 years old, was admitted to the Faulkner Hospital on the afternoon of March 21, 1943. She had been in good health until the evening of March 18 when she had a rather abrupt onset of malaise, fever, restlessness, and insomnia. On the following day she had a slight sore throat with dysphagia and a harsh unproductive cough. On the afternoon of March 20 her temperature was 99.6° F. and her throat was very sore and slightly swollen but did not appear inflamed. At that time she began to have substernal distress with her cough. That night she became more restless. On the following morning the temperature was 100.6° F., pulse was 90, respirations were 28, and the patient was slightly cyanotic, somewhat stuporous, and complained of substernal oppression. That evening her temperature was 103.6° F.; pulse, 120; respirations, 42. The breath sounds in her lungs were diminished and a few râles were heard at both lung bases, but there were no signs of consolidation. She was sent to the hospital immediately.

On admission, there was slight injection of the pharynx, some hoarseness, and slight tenderness over the larynx. Examination of the lungs was negative except for a few scattered râles. The leukocyte count was 4,000 with a predominance of lymphocytes. Blood culture showed no growth. Urine was concentrated and showed a trace of albumin and a few white blood cells in the sediment.

The patient was given oxygen, sedation, and a total of 4 gm. of sufadiazine. She became increasingly cyanotic and her respirations more labored. She also com-

\* Courtesy of Drs. A. A. Cushing and G. K. Mallory.

plained of more pain throughout the chest. The respirations sounded "moist" but there was very little cough or sputum. During the night, tracheal râles increased, and signs of consolidation and many râles were made out in the right lower chest. She died 12 hours after entry.

### Post-Mortem Examination

Autopsy was performed 2 hours following death. The right pleural cavity contained approximately 100 cc., and the left approximately 300 cc. of serosanguineous fluid. The heart was negative.

The right lung weighed 740 gm. and the left, 1100 gm. The pleural surfaces of both lungs were smooth and glistening. The right lung anteriorly was salmon pink, soft and pillowy in consistence, and showed discernible alveolar detail associated with early emphysematous changes. There were three areas, 1 to 2 cm. in diameter, deep red and sharply demarcated from the surrounding lung tissue. Two of these were in the upper lobe and one in the middle lobe. These areas were subcrepitant and on section had a homogeneous red-purple color and exuded fluid blood on pressure. The posterior half of the right lung was boggy and subcrepitant to noncrepitant. The color was dark red to violaceous and was mottled by irregular areas of deeper purple-red. On section the cut surface was wet, oozed considerable blood, and presented irregular and rounded areas of deep purple, noncrepitant tissue from which dark, bloody fluid could be expressed. These areas bore no relationship to bronchi or bronchioles. The entire left lung had the same appearance externally and on section as the posterior half of the right lung. No pink, crepitant tissue was visible and both the upper and lower lobes had the consistence of liver tissue, although somewhat more friable and nodular. The trachea and major bronchi were filled with frothy fluid. The mucous membrane from the trachea to the tertiary bronchi was covered by a thin, yellow-gray, membranous exudate which could be peeled away only with difficulty to reveal an intensely congested, raw surface.

The spleen weighed 125 gm. and was not remarkable. The liver weighed 1700 gm. and appeared negative.

*Microscopic Examination.* In the lungs some alveoli were empty and appeared distended. Others contained edema fluid while still others contained red blood cells and varying numbers of polymorphonuclear leukocytes. In such alveoli there were masses of cocci. Others were filled with polymorphonuclear leukocytes and cocci. In addition, several small abscesses were present. Some of the alveolar capillaries were thrombosed. The bronchioles showed necrosis of their epithelium and of the underlying connective tissue which was infiltrated with numerous cells, some of which were polymorphonuclear

leukocytes, but many of which were so necrotic that their type could not be distinguished. The blood vessels of the bronchioles contained fibrin in their walls and some were thrombosed. The majority of the bronchioles were empty but a few contained red blood cells or polymorphonuclear leukocytes and cocci. There was a slight perivascular infiltration of some lymphocytes and plasma cells. Several medium-sized arteries contained thrombi. The pleura was edematous and the pleural lymphatics contained polymorphonuclear leukocytes and large mononuclear cells.

The epithelium of the trachea had been completely destroyed (Fig. 6). There was extensive necrosis involving the connective tissue of the wall of the trachea and of the leukocytes which had infiltrated it. Masses of cocci were present here. There were focal hemorrhages and deposits of fibrin. The majority of the blood vessels were partially or completely thrombosed. There was a marked infiltration of lymphocytes and plasma cells around the glands and between the smooth muscle fibers.

The heart was negative. In the spleen the histiocytes of the malpighian corpuscles were hyperplastic. The bone marrow showed maturation arrest of the granulocytic series, the majority of the cells being myelocytes with only an occasional adult polymorphonuclear leukocyte present.

*Bacteriology.* A hemolytic *Staphylococcus aureus* was obtained in pure culture from the heart's blood, lungs, right main bronchus, pleural cavities, and spleen.

*Virus Studies.* A filtrate of a 20 per cent suspension of the left upper lobe of the lung obtained at autopsy was used for virus studies. In the first attempt no lesions were obtained after ten intranasal passages in mice. Lungs of the third mouse passage, however, yielded virus when transmitted through eggs by allantoic inoculation. Allantoic fluid from subsequent egg passages produced fatal lesions in mice and agglutinated hen cells to a dilution of 1:256. This agglutination was inhibited by anti-PR 8 rabbit serum to a titer of 1:2048 and by anti-Lee serum to a titer of 1:8. Comparable inhibitions were obtained with PR 8 virus. Serum obtained from cardiac blood at autopsy gave no significant titer of antibodies for either PR 8 or Lee virus in complement fixation or agglutination inhibition tests.

### Case 2

Case 2 has been reported elsewhere<sup>6</sup> because of the finding of acute nonbacterial myocarditis. The patient was a woman, 34 years of age, who had been in good health until the middle of December, 1942, when she had a mild attack of bronchitis or "flu." Cough and fatigue persisted thereafter but there were no positive

findings demonstrable by physical or roentgenological examination. On April 4, 1943, the patient noticed unusual fatigue and on the following morning she had generalized malaise and an increase in her cough which was unproductive. On April 6 she had chilly sensations, and on that evening her temperature was 103.4° F. but her lungs were still clear. On April 9 her condition became much worse; the temperature was 102.6° F.; pulse was 124, feeble and thready, and the blood pressure was 125/78 mm. Hg. There were suggestive signs of consolidation of the right lower lobe and a few scattered râles in both lung bases. Heart sounds were faint and distant. The white blood count was 20,000, of which 70 per cent were polymorphonuclear leukocytes. The urine showed 2 plus albumin and a few white blood cells. Sputum could not be obtained but a throat culture yielded a scant growth of *Neisseria catarrhalis* and a few colonies of *Staphylococcus aureus*.

The patient was given 4 gm. of sulfadiazine and 1 gm. every 4 hours thereafter. She received a total of 8 or 9 gm. The signs and symptoms thereafter were those of increasing cardiac embarrassment and she was admitted to the Peter Bent Brigham Hospital late in the afternoon of April 10. A bedside roentgenogram of her chest at that time showed irregular mottling extending out from both hilar regions and a small amount of fluid in the axillary region. An electrocardiogram showed complete heart block and bizarre ventricular complexes. In spite of oxygen therapy and digitalis, the patient continued a downhill course and died 7 hours after admission.

### Post-Mortem Examination

Autopsy was performed 8 hours after death. The right pleural cavity contained 950 cc. of a pale yellow fluid with flecks of fibrin, and the left contained 600 cc. of a similar fluid. There were several old fibrous adhesions binding the anterior and lateral surfaces of the right lung to the chest wall. Elsewhere the pleural surfaces of both lungs were smooth and glistening. The heart weighed 480 gm. The myocardium was light pink. Both the right and left ventricles were increased in thickness, the right measuring 0.8 and the left 2.0 cm. The valves were negative.

The lungs were somewhat heavier than normal. They were fairly crepitant throughout save at the bases where diminution in crepitation was more marked. Bloody fluid could be expressed from the cut surfaces. No areas of consolidation were present. The bronchial mucosa was slightly reddened but was glistening and free from exudate. The trachea appeared normal. There was no secretion or exudate present.

The spleen weighed 120 gm. Its architecture was well preserved and only a small amount of pulp could be scraped away. The liver weighed 1700 gm. and was negative. Bone marrow expressed from a rib was copious and deep red.

*Microscopic Examination.* The lungs showed a considerable degree of congestion. The alveoli contained a moderate number of large mononuclear cells, many of which had phagocytized carbon. Sections from the right lower lobe showed a perivascular infiltration of a few lymphocytes, plasma cells, eosinophils, and an occasional mast cell



(Fig. 2). One focus of acute inflammation was found in a section from this lobe (Fig. 1). Here several alveoli contained fibrin, large mononuclear cells, lymphocytes, and polymorphonuclear leukocytes. The walls of these alveoli contained cells of the same type. There was also swelling of the alveolar lining cells. A section from the left upper lobe likewise showed an acute inflammatory lesion in which the alveoli contained polymorphonuclear leukocytes, fibrin, and some large mononuclear cells. Stains for bacteria failed to reveal any microorganisms in these acute lesions or elsewhere. The epithelium of the bronchioles was unaffected. Their walls were infiltrated with a few lymphocytes, plasma cells, and a rare polymorphonuclear leukocyte. The heart showed extensive necrosis of muscle fibers with interstitial infiltration of lymphocytes, plasma cells, some large mononuclear cells, and an occasional eosinophil and mast cell. The spleen showed hyperplasia of the histiocytes in the malpighian corpuscles. In some areas of the liver there was central necrosis. The hepatic cells throughout showed fatty as well as hydropic degeneration. The bone marrow was not remarkable.

*Bacteriology.* Cultures of the heart's blood, lungs, and serous cavities yielded no growth.

*Virus Studies.* A Berkefeld V filtrate of a 20 per cent suspension of part of the right lower lobe was used for virus studies. The first attempt in mice was abandoned after six intranasal passages failed to produce pulmonary lesions. In the second attempt a different portion of the same lung was used, lesions appeared in the second passage, and death occurred on the fourth and subsequent passages. Survivors of the third passage were later given a challenge dose of 100 lethal doses of PR 8 and survived. In eggs, the virus became well established by the sixth passage. The allantoic fluid of later passages agglutinated hen cells up to a dilution of 1:2048. This agglutination was inhibited by anti-PR 8 rabbit serum to a titer of 1:1024, and by anti-Lee rabbit serum to a titer of 1:32. The patient's own serum obtained before death failed to fix complement or inhibit hen cell agglutination with PR 8 virus.

### Case 3 \*

The patient was a white, single office worker, 18 years old, who was admitted to the Evans Memorial Hospital at 4:00 p.m. on December 13, 1943. Her illness had begun abruptly at 3:00 p.m. on December 11, when she noted a sore throat and dysphagia. On the following day a physician found her temperature to be 101° F. and told her she had a bad sore throat and prescribed cough medicine. She had anorexia and vomiting that day and felt somewhat drowsy. At 1:00 a.m. on the morning of admission she developed severe pains across her lower chest and a slight cough productive of a small amount of sputum. She was given four sulfonamide

\* Courtesy of Drs. Chester S. Keefer and John J. Curry.

tablets but soon became delirious and her physician sent her into the hospital after making a diagnosis of pneumonia.

On admission she appeared critically ill, toxic, cyanotic, delirious, dyspneic, and semicomatose. She was unresponsive, and incontinent of urine and feces. Temperature was 106.6° F. (rectal); pulse, 175; respirations, 53; blood pressure, 60/40 mm. Hg. She was well developed but poorly nourished. Her skin was hot and dry and she was markedly dehydrated. The mouth and tongue were very dry with thick, yellow, ropey material at the base of her tongue. Her pharynx was deeply injected. There was slight dullness at the base of the right lung posteriorly where the breath sounds were suppressed. A few fine, crepitant râles were heard there and in the right posterior axillary region. The lungs were otherwise clear. The heart sounds were rapid, faint, and distant. The hemoglobin was 10 gm.; red blood count, 4.86 million; white blood count, 1,350, with 24 per cent polymorphonuclear leukocytes, 72 per cent lymphocytes, and 4 per cent monocytes. A throat smear showed gram-positive diplococci and culture of the sputum yielded beta hemolytic streptococci and hemolytic *Staphylococcus aureus*. The latter also was grown from the blood culture. A portable roentgenogram of the chest showed large patches of dense infiltration in all but the upper part of the right lung field and a few similar patches were noted in the left mid-lung field.

Oxygen therapy was started at the time of entry and the patient was given an intravenous infusion of 1200 cc. of 5 per cent glucose in saline solution to which 5 gm. of sodium sulfadiazine had been added. The cyanosis improved somewhat and the blood pressure rose temporarily to 90/60 mm. Hg. The patient remained stuporous and combative. The râles in her chest increased and loud tracheal rhonchi were heard. The blood pressure soon dropped again and the pulse became thready and imperceptible. She became extremely restless and had a slight convulsive seizure with muscular twitchings of her face. Bloody foam exuded from her mouth and she expired about 3 hours after admission.

### Post-Mortem Examination

Autopsy was performed 3 hours following death. The mucosa of the oropharynx and larynx was markedly reddened. Each pleural cavity contained approximately 500 cc. of slightly turbid, yellow, watery fluid. The pleural surfaces were smooth, dull, and bright red-gray. The heart weighed 225 gm. and was negative.

The right lung weighed 900 gm. and the left, 730 gm. The lungs were soft, boggy, and subcrepitant with only a few slightly firmer areas suggesting consolidation. On section, the alveolar architecture was obscured by a dark crimson background from which a large amount of frothy, serosanguineous fluid could be expressed. No definite consolidation could be found. The trachea and bronchi, which were filled with frothy, serosanguineous fluid, were lined with a dull, dark crimson mucosa which appeared to be extensively and superficially eroded. The hilar lymph nodes were enlarged and soft.

The spleen weighed 140 gm. The malpighian corpuscles were well defined against a dark crimson background. A large amount of soft pulp could be scraped away. The liver weighed 1280 gm. and was not remarkable.

*Microscopic Examination.* Sections from the lungs showed a variety of changes. In some there was intense congestion and the alveoli contained edema fluid, red blood cells, a few large mononuclear cells, and a rare polymorphonuclear leukocyte. In addition, there was a hyaline membrane, often appearing somewhat fragmented, lining some of the alveolar ducts and alveoli. The bronchioles in these areas contained edema fluid, numerous red blood cells, and large mononuclear cells. In addition, there was abscess formation. At the periphery of the abscesses, the intra-alveolar hemorrhages were more extensive and there was also a deposit of fibrin. The blood vessels here contained thrombi. Some bronchioles showed necrosis of their epithelium and contained in their lumina polymorphonuclear leukocytes, large mononuclear cells, red blood cells, and cocci. The walls of the bronchioles were infiltrated with large mononuclear cells and polymorphonuclear leukocytes. Gram-positive cocci, growing both in clusters and in long chains, occurred in all sections but were especially numerous in the abscesses and in the bronchioles that showed necrosis of their mucosa. In some sections the pleura was covered with a thin layer of fibrin.

The heart showed a few scattered, large mononuclear cells and lymphocytes in the interstitial tissue of the myocardium. In the adrenal cortex there were several foci of necrosis in which the necrotic cells had been invaded by polymorphonuclear leukocytes. The bone marrow showed numerous myelocytes but only rare adult granulocytes.

*Bacteriology.* Hemolytic *Staphylococcus aureus* and *Streptococcus hemolyticus* were cultured from the pericardial cavity, the upper and lower lobes of the right lung, the lower lobe of the left lung, the right and left bronchi and both pleural cavities.

*Virus Studies.* A 20 per cent suspension of a portion of the hemorrhagic lung was passed through a Berkefeld V filter and the filtrate used for inoculation of mice and of chick embryos. No transmissible lesions were found in the mice after six intranasal passages, and further attempts were abandoned. The first intra-allantoic inoculation, however, yielded virus recognizable by moderate agglutination of the erythrocytes of the embryo as the allantoic fluid was withdrawn. Fifth passage allantoic fluid, diluted 1:10, regularly produced deaths with typical lesions in mice, and these were completely prevented by simultaneous inoculation of anti-influenza A (PR 8) ferret serum but were unaffected by similar amounts of anti-influenza B (Lee) ferret serum. This virus behaved in an atypical manner in agglutination-inhibition tests. With anti-PR 8 rabbit serum inhibition occurred in a titer of 1:64, and with anti-Lee serum in a titer of 1:32. Further studies of this virus are in progress.

### Case 4

Case 4 was also included in a previous report<sup>6</sup> because of small myocardial lesions found at autopsy. The patient was an Italian iron worker, 39 years of age, who was admitted to the Boston City Hospital on January 31, 1944, complaining of dyspnea and hemoptysis of 2 days' duration. He denied having had cardio-respiratory symptoms until January 23 when he had a slight "head cold" which was followed by malaise, anorexia, and prostration. On January 26, he had severe shaking chills followed by fever. Three days later he began to have marked dyspnea, cyanosis, and cough, and raised grossly bloody sputum. He also had two attacks of substernal pain.

When he arrived at the hospital the patient was markedly cyanotic and dyspneic and had audible tracheal râles. He was coughing and expectorating dark red blood. The temperature was 98.4° F., pulse was 100 and regular, and respirations were 28 and labored and had a prolonged expiratory phase. The blood pressure was 138/88 mm. Hg. In both lungs there were numerous medium and coarse, moist râles but no definite signs of consolidation. The leukocyte count was 19,000 of which 88 per cent were polymorphonuclear leukocytes, and the hemoglobin was 96 per cent. The nonprotein nitrogen of the blood was 135 mg. per 100 cc. A smear of sputum showed gram-positive cocci and bacilli. An electrocardiogram showed left axis deviation but no other significant abnormality. A bedside roentgenogram of the chest showed diffuse clouding of both lung fields with irregular, fluffy areas of density. The patient failed to respond to therapy with oxygen and other supportive measures and died 14 hours after entry.

### Post-Mortem Examination

Autopsy was performed 14 hours after death. The surfaces of the pleural cavities were smooth and glistening. The right cavity contained 600 cc. of a reddish, straw-colored fluid; the left, 500 cc. of a similar fluid. The heart weighed 330 gm. The coronary arteries showed atheromatous changes but were not occluded. There was a fibrous scar about 2 cm. in diameter in the interventricular septum.

The right lung weighed 1250 gm. and the left, 1100 gm. Their surfaces were glistening and transparent. Both lungs were subcrepitant throughout. On section, no discrete areas of consolidation were present but all lobes felt much firmer than normal. The trachea and bronchi contained a slightly mucoid, serosanguineous fluid.

The spleen weighed 105 gm. The cut surface was purplish red and the malpighian corpuscles were poorly delineated. The liver weighed 1690 gm. and cut with some increased resistance. The kidneys each weighed 205 gm. and were not remarkable save for a moderate degree of congestion.

*Microscopic Examination.* The heart showed large areas of scarring of the myocardium. In addition, there was necrosis of scattered muscle fibers and an interstitial infiltration of large mononuclear cells, lymphocytes, plasma cells, eosinophils, and polymorphonuclear leukocytes.

All lobes of the lungs showed essentially the same histologic picture. Some alveoli contained large mononuclear cells, many of which had

phagocytized carbon. In other alveoli there was edema fluid, a few red blood cells, delicate strands of fibrin, and varying numbers of polymorphonuclear leukocytes (Fig. 4). Numerous alveolar ducts and alveoli were lined with a dense, acidophilic, hyaline membrane in which were often embedded particles of carbon, polymorphonuclear leukocytes, and large mononuclear cells (Fig. 3). Some of the ducts and alveoli lined with this membrane were empty; others contained edema fluid and strands of fibrin. There were a moderate number of alveoli in foci which contained polymorphonuclear leukocytes, large mononuclear cells, and fibrin. The alveolar capillaries contained an increased number of polymorphonuclear leukocytes, especially those adjacent to the hyaline membrane. Thrombosis of the capillaries was seen occasionally. The epithelium of the bronchioles was intact. Some of the bronchioles were empty; others contained mucus, polymorphonuclear leukocytes, red blood cells, and fibrin. Their walls were infiltrated with fairly numerous polymorphonuclear leukocytes, lymphocytes, a few plasma cells, and a rare mast cell. The septa were markedly edematous and their lymphatics were dilated. Some of the septa were infiltrated with large mononuclear cells, lymphocytes, plasma cells, and polymorphonuclear leukocytes. Stains for bacteria failed to reveal microorganisms in any section.

The spleen showed in the larger veins a subendothelial infiltration of a few lymphocytes and plasma cells. The liver cells in the centers of the lobules had been replaced by large mononuclear cells, lymphocytes, and a few eosinophils. In the kidneys, some tubules contained necrotic epithelial cells in their lumina and such tubules were lined with flattened epithelium in which an occasional mitotic figure was present. There was an interstitial infiltration of lymphocytes, plasma cells, and eosinophils. The bone marrow was essentially normal.

*Bacteriology.* Cultures of the various lobes of the lungs showed no growth. An unidentified gram-negative diplobacillus was cultured from the heart's blood and a gram-negative diplococcus from the spleen. Both of these organisms were considered to be contaminants.

*Virus Studies.* A sterile filtrate of a 20 per cent suspension of some hemorrhagic lung tissue was used for virus studies. Typical lesions appeared in mice on the sixth intranasal passage and deaths occurred regularly beginning with the seventh. Neutralization tests with the mouse lung suspensions and immune ferret serums showed definite protection by anti-PR 8 serum and none by anti-Lee serum. Virus was recognized in the allantoic fluid of the first eggs inoculated with the filtered lung suspension. The fluid from the ninth passage agglutinated hen cells up to a dilution of 1:512 and this agglutination was inhibited

by anti-PR 8 ferret serum to a titer of 1:1024 and by anti-Lee serum to a titer of 1:64. Serum obtained from the patient before death had no significant antibodies for the PR 8 or Lee viruses.

### *Case 5*

A white painter, 61 years old, was admitted to the Boston City Hospital on March 6, 1944, too ill to answer questions. From his wife and sister it was learned that he had always been in good health except for childhood diseases and an attack of rheumatic fever in 1902, from which he recovered without recurrences or sequellae. For 2 months preceding the present illness he had seemed unusually tired but had improved and felt perfectly well during the week prior to entry. On March 3, at 3:00 p.m., while at work he had a shaking chill and went home feeling feverish and weak. He went to bed and on the following day had chilly sensations, pleuritic pain in the right lower chest, and cough productive of large amounts of dark, rusty sputum. A physician found his temperature on that afternoon to be 103° F. and diagnosed "grippe." His cough and chest pain increased in severity and on the morning of admission he was having drenching sweats.

On admission he appeared severely ill, markedly dyspneic, cyanotic, and dehydrated. His temperature was 103° F.; pulse, 136; respirations, 42; blood pressure, 158/78 mm. Hg. He was coughing and raising rusty sputum and obviously having pain with respiration. The throat was injected and covered with a mucoid exudate. Respiratory movements were limited, particularly on the right. There was dullness to flatness over the right lower lung posteriorly and crepitant râles were heard over this area and in the right axilla, but the rest of the lungs seemed clear. The heart sounds were rapid but regular. Hemoglobin was 95 per cent; white blood count, 2,000, with 60 per cent polymorphonuclear leukocytes, many of them band forms. Type I pneumococci were identified in his sputum by the Neufeld method and the same organism was obtained from the blood cultures. The urine was concentrated and showed 4 plus albumin, occasional white blood cells, and numerous granular casts. The nonprotein nitrogen of the blood was 45 mg. per 100 cc. and the Hinton test on the blood was negative.

Oxygen therapy was begun on admission and an intravenous infusion of 15,000 cc. of saline solution containing 5 gm. of sodium sulfapyrazine was given. Anti-pneumococcus serum was also given intravenously in amounts of 1, 5, and 14 cc. at approximately 2-hour intervals, a total of 200,000 units being given between 5 and 11 p.m. There were no immediate untoward effects and there was a slight decline in the temperature and pulse rate during this treatment. About 1 hour after the last dose, however, the patient's condition became very poor, the blood pressure dropped rapidly despite coramine and caffeine, and he died about 40 minutes later.

### *Post-Mortem Examination*

Autopsy was performed 9 hours following death. The right pleural cavity contained approximately 500 cc. of cloudy, yellow fluid. There were friable, fibrinous adhesions to the whole lower lobe and to the diaphragm. The left pleural cavity contained no excess fluid and the pleural surfaces were smooth and glistening.

The heart weighed 400 gm. and showed changes consistent with arteriosclerotic heart disease.

The right lung weighed 1910 gm. The upper and middle lobes were deep red while the lower lobe was yellow. The consistence of the lower

lobe was firm and noncrepitant. On section, a yellow-pink material gushed forth. The cut surface was roughened and showed no discrete, firm, or raised areas. The bronchioles exuded a yellow, purulent material on pressure. The upper and middle lobes were crepitant. On section, pink, watery fluid exuded. The cut surface was uniformly soft and wet. The bronchioles contained pink, watery fluid. The left lung weighed 820 gm. It resembled the upper and middle lobes of the right lung, both externally and on section. The trachea and major bronchi contained a watery, pink, frothy fluid. The mucosa of the trachea and bronchi was injected but was intact. The spleen weighed 265 gm. and was soft. The liver was slightly enlarged, weighing 2100 gm.

*Microscopic Examination.* In the heart there were some focal collections of large mononuclear cells in the interstitial tissue of the myocardium.

In the lungs, sections of the right upper lobe showed the majority of the alveoli to contain edema fluid and a varying number of carbon-filled, large mononuclear cells. Some alveoli were considerably distended. The epithelium of the bronchioles was intact (Fig. 5). Some bronchioles were empty; others contained edema fluid. In sections of the right lower lobe the alveoli contained some large mononuclear cells and numerous polymorphonuclear leukocytes, many of which were necrotic. Many alveoli also contained fibrin adjacent to their walls, while there was little or none in the central portions of their lumina. In places, the alveolar capillaries were congested; in others they were thrombosed. The pleura was covered with a layer of fibrin beneath which were fairly numerous polymorphonuclear leukocytes. The left upper lobe was essentially negative save for some distended alveoli. The left lower lobe was similar to the right upper lobe. In addition, several alveoli contained a few polymorphonuclear leukocytes, large mononuclear cells, and a small amount of fibrin. The epithelium of the bronchioles was intact. Their walls were infiltrated with numerous lymphocytes and plasma cells. Their lumina contained edema fluid and a few polymorphonuclear leukocytes. Numerous gram-positive diplococci were present in the alveoli of the upper and lower lobe of the right lung and in the lower lobe of the left. In the upper lobe of the left lung similar microorganisms were seen only in the alveolar capillaries.

The bone marrow revealed numerous myelocytes but only rare adult leukocytes.

*Bacteriology.* Pneumococcus, type I, was grown from the heart's blood and from all lobes of the right lung, and the lower left lobe.

*Virus Studies.* A portion of the right upper lobe was taken under

sterile precautions and preserved at  $-70^{\circ}$  C. for virus studies. A 20 per cent suspension of sterile filtrate was used for intranasal inoculation of mice and intra-allantoic inoculation of chick embryos. Lesions appeared in the lungs of mice on the second passage and deaths occurred in the third and subsequent passages. There was slight agglutination of the embryonic erythrocytes in the allantoic fluid of the first egg passage and strong agglutination in subsequent passages. In a preliminary neutralization test the virus produced fatal lesions regularly in mice and these were prevented by the use of anti-influenza A ferret serum diluted 1:50 but not by similar amounts of anti-influenza B serum. Serum obtained from the patient on admission was used in agglutination-inhibition tests and gave a titer of 1:64 with influenza A (PR 8), 1:256 with influenza B (Lee), and 1:256 with the patient's own virus.

#### SUMMARY OF PATHOLOGIC FINDINGS

It will be noted from the above data that two of the cases (2 and 4) were uncomplicated by bacterial infections. In case 2, death resulted from cardiac failure associated with an extensive acute myocarditis. The changes in the lungs were surprisingly slight and consisted of acute lesions involving a few alveoli. The bronchioles were unaffected. In case 4, death was due to the pulmonary involvement. The lungs in this case showed edema, some alveolar hemorrhages, fibrin, and extensive formation of a hyaline membrane. The epithelium of the bronchioles was intact but their walls were infiltrated with cells of various types. Their lumina were empty or contained mucus, leukocytes, red blood cells, and fibrin. There was marked edema of the septa.

Case 1 was complicated by a fulminating *Staphylococcus aureus* infection, causing an extensive necrotizing process involving the trachea, bronchi, and bronchioles. The alveoli showed edema, hemorrhages, an exudate of polymorphonuclear leukocytes, and abscess formation.

In case 3 an attack of influenza was complicated by a secondary infection with a beta hemolytic streptococcus and a hemolytic *Staphylococcus aureus*. The lungs showed alveolar hemorrhages, edema, and hyaline membrane formation in some sections. In others there was abscess formation. The epithelium of the bronchioles was intact in the former areas and necrotic in the latter. There was in addition an acute fibrinous pleuritis.

Case 5 was complicated by a pneumococcal pneumonia of the right lower lobe and a pneumococcal bacteremia. The right lower lobe showed a resolving lobar pneumonia. The right upper and left lower lobes showed edema and, in addition, in the left lower lobe several alveoli contained polymorphonuclear leukocytes, large mononuclear



cells, and fibrin. The bronchiolar epithelium in all lobes was intact. Bronchiolar walls were infiltrated with lymphocytes and plasma cells. There was an acute fibrinous pleuritis of the right lower lobe.

#### CORRELATION OF PATHOLOGIC CHANGES AND VIRUS STUDIES

In each case, material from only one lobe was utilized for the isolation of the virus. In case 1, unfortunately no note was made as to which lobes the microscopic sections represented. In case 2, the virus was isolated from the right upper lobe and histologically the only lesions present were focal lesions involving a few alveoli and consisting of an exudate of polymorphonuclear leukocytes, fibrin, and some large mononuclear cells. In case 3, no record was kept as to which lobe was studied for the presence of a virus. In case 4, likewise, no such record was kept, but the process was uniform throughout all lobes and it would seem justifiable to assume that the changes described, namely, edema, alveolar hemorrhages, fibrin, and hyaline membrane formation, represent the reaction to the virus. It should be noted that the bronchiolar epithelium was intact in this case as it was in case 2. In case 5, virus was isolated from the right upper lobe and sections from this lobe showed edema of the alveoli with an exudate of a moderate number of large mononuclear cells. As in cases 2 and 4, the bronchiolar epithelium was unaffected.

The fact that a virus was found in a single lobe in each instance is, of course, no indication that it was not present in some, if not all, of the other lobes. However, due to practical difficulties it was impossible to utilize more than one lobe from each case for virus studies.

#### COMMENT

As was indicated earlier in this paper, the number of fatal cases in which influenza virus has been demonstrated and the pathologic changes described is remarkably small. Only four cases with pathologic descriptions of the lungs have been found by us in the literature. We have had an opportunity to examine five additional cases and these form the basis of this report.

It appears of no value to discuss the pathologic changes which have been described in previous pandemics and epidemics of influenza for nothing is known as to the etiologic agent. With a very rare exception, all such cases were complicated by secondary bacterial infections and the pathologic lesions described were caused for the most part, if not entirely, by such secondary invaders. Goodpasture<sup>7</sup> described two cases which were bacteria free. His first patient died 7 days after the

initial symptoms and 2 days after signs of consolidation appeared in the lungs. Microscopic examination of the lungs showed injury and destruction of the alveolar walls with hemorrhage, edema, a little fibrin, and scant cellular exudate. The alveolar ducts were dilated and some of them showed a hyaline membrane on their walls. His second case was of a subacute type with a terminal exacerbation. Microscopically, the lungs showed alveolar hemorrhages, innumerable foci of polymorphonuclear leukocytes, fibrin, large mononuclear cells, disintegrating hyaline material, and small areas of necrosis of the alveolar walls. In some areas there was a thick layer of hyaline material on the walls of dilated ducts and alveoli. The epithelial lining of the large and small bronchi was intact. In certain respects these two cases resemble histologically our case 4 which was likewise bacteria free.

In our series, two cases were bacteria free and three were complicated by secondary bacterial infections.

Much emphasis has been placed in the past on necrotizing bronchiolitis as a feature of influenza. Such a process also has been found in experimental infections with influenza virus in mice and ferrets. However, in our two cases which were not complicated by bacteria the epithelium of the bronchioles was unaffected. This was also true of Goodpasture's case<sup>7</sup> in which he described the bronchioles. Furthermore, in our cases complicated by secondary bacterial invaders, the bronchioles in the portions of the lung which were not involved by the bacterial infection but which contained the virus were unaffected.

From our series of cases it would seem that it would be difficult to recognize changes produced by the virus in the presence of bacterial infections. It is possible that more definite lesions due to the virus had not been produced because of the short duration of the disease in these cases—2 to 3 days. It will be noted from the descriptions of the histologic changes in our cases that the lesions in four of the five cases were minimal. However, it is entirely possible that if it had been practicable to make multiple sections of each lobe, more severe lesions might have been found. A similar situation was true with the virus studies. In each case, tissue from only one lobe was tested for the presence of virus. Another explanation of the lack of severity of the lesions is the short course of the disease in the three cases complicated by secondary bacterial invaders. Death in these cases may well have been due primarily to the bacterial infections. In case 2, in which the pulmonary lesions were minimal, the duration of the disease was probably 7 days and death was due to acute myocarditis. The lesions in this case may represent a minimal infection with virus or possibly a late stage. The

TABLE I  
Certain Relevant Data in Five Cases in Which Influenza Virus Was Recovered from the Lungs

Case number	1	2	3	4	5
Sex and age (years)	Female, 26	Female, 34	Female, 18	Male, 39	Male, 61
Dates:					
Admission	3/21/43; 9 P.M.	4/10/43; 6 P.M.	12/13/43; 4 P.M.	1/31/44; 4 P.M.	3/6/44; 3 P.M.
Onset of influenza	3/18; P.M.	4/4; P.M.	12/11; 3 P.M.	1/23	3/3; P.M.
Onset of pneumonia	3/20; P.M.	4/6 or 4/9(?)	12/13(?); 1 A.M.	1/26 or 1/29	3/4
Death	3/22; 8 A.M.	4/11; 1 A.M.	12/13; 7 P.M.	2/1; 1 A.M.	3/7
Pulmonary involvement					
Clinical: Consolidation	R.L.	R.L.(?)	Patchy R. and L.	°	R.L.
Râles	Bilateral	R.L., L.L.	Bilateral	Bilateral	R.L., m.
X-ray: Consolidation		°	°	°	
Mottled density		Mid-lungs	Bilateral	Bilateral	
White blood cell count					
Number per cmm.	4,000	20,000	1,350	19,000	2,000
% polymorphonuclear cells	40	70	24	88	60
Bacteriology					
Sputum			S. au.; Str. B	Negative	Pn. I
Blood (ante-mortem)	No growth	No growth	S. au.	No growth	Pn. I
Cardiac blood (autopsy)	S. au.	No growth	No growth	G-Bact. (contam.)	Pn. I
Lungs (autopsy)	S. au.	No growth	Str. B; S. au.	No growth	Pn. I
Others (autopsy)	P.F.; S. au.	P.F.; no growth	P.F.; Str. B. and S. au.		P.F.; Pn. I
	Bronchus; S. au.		Bronchus; St. B. and S. au.		
Virus isolation					
Source	Lung (Lu)	Lung (R.L.)	Lung	Lung	Lung (R.u.)
Result in mice	Influenza A	Influenza A	Negative	Influenza A	Influenza A
Result in chick embryos	Influenza A*	Influenza A	Influenza A(?)	Influenza A	Influenza A

Abbreviations: R. = right; L. = left; l. = lower; m. = middle; u. = upper lobe.

S. au. = Hemolytic *Staphylococcus aureus*; Str. B. = Beta hemolytic streptococci.

Pn. I = Type I pneumococcus; P.F. = pleural fluid; G- = gram negative.

\* Obtained by allantoic inoculation of a suspension of lung from the third mouse passage in this case. Others obtained from direct inoculation of the eggs with original lung suspensions.

fourth patient (case 4) lived 9 days and died of pulmonary involvement due to virus alone. The histologic changes, in our opinion, represent the typical picture of a pure influenza virus pneumonia.

Some of the relevant data in the five cases are summarized in Table I. It is seen that the three cases infected with bacteria showed a marked leukopenia. In these cases the bone marrows show maturation arrest of the granulocytic series. In the two bacteria-free cases, leukocytosis was present and the bone marrows were normal. The leukopenia may be attributed to the short course of the disease or to a depressant action of the bacteria. However, it appears that the uncomplicated influenza virus infections were accompanied by leukocytosis rather than leukopenia.

Because of the difficulties usually encountered in isolating an influenza virus from fatal cases as compared with the relative ease with which they were obtained by mouse and chick embryo inoculation and by passage from these cases and from other non-fatal cases,<sup>8</sup> the possibility must be considered that the viruses isolated from the present cases may not have originated from these lungs but have occurred as laboratory contaminants.<sup>9</sup> Such a possibility is extremely unlikely. The viruses in the first two cases were each isolated at a time when no other influenza virus was available in the same laboratory. In the case of the other strains, evidence for the presence of the virus was obtained after the original allantoic inoculation in each instance, and then increased with further passages. Mouse inoculation and passage of the same lung suspensions were successful in only two of the three cases. Furthermore, the virus in one of these cases was serologically distinct from the others. In addition, unsuccessful attempts were made by similar passages in mice and eggs obtained from the same sources to isolate viruses from nine other fatal cases of influenza and atypical pneumonia.

#### SUMMARY

1. The pathologic changes have been described in five cases in which influenza virus was obtained from the lungs. Only three earlier reports in which the structural changes were described have been found in the literature.

2. Two of our cases were bacteria free; the other three had secondary bacterial invaders.

3. One of the bacteria-free cases showed pathologic changes which were considered typical of influenzal pneumonitis. These consisted of edema, alveolar hemorrhages, fibrin, and the formation of a hyaline membrane.

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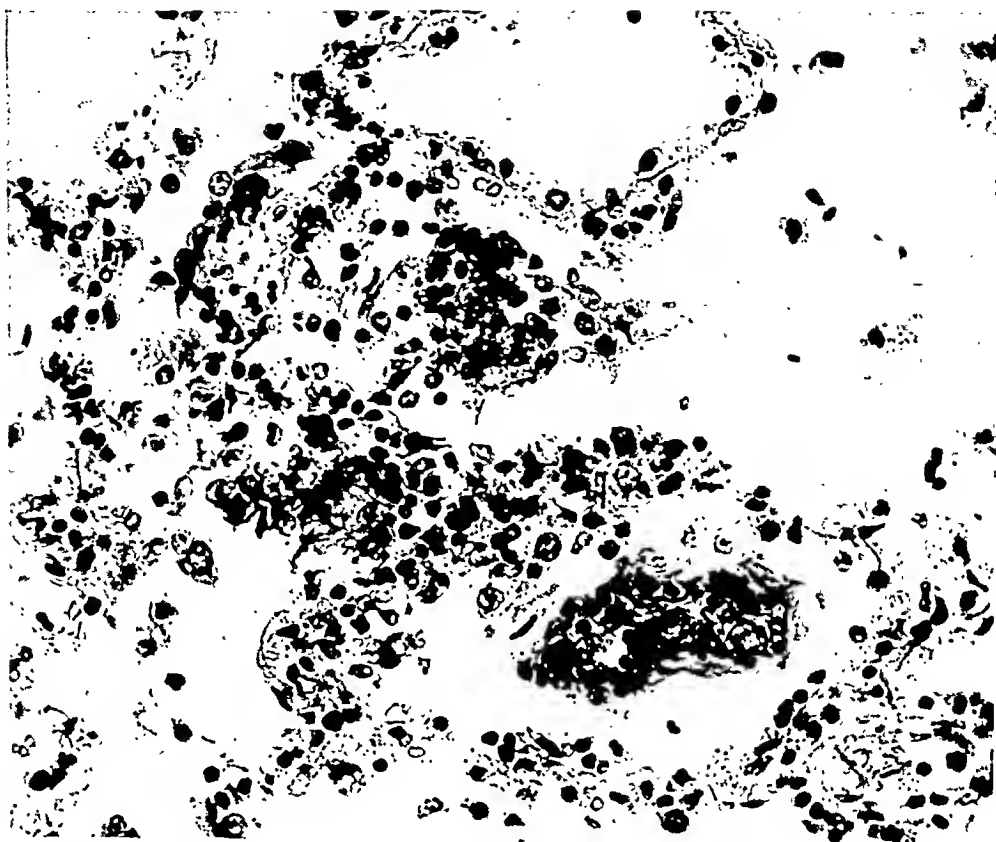
## DESCRIPTION OF PLATES

## PLATE 153

FIG. 1. Case 2. Acute focal lesion in the right lower lobe. The alveoli contain fibrin, large mononuclear cells, lymphocytes, and polymorphonuclear leukocytes. There is also swelling of the cells lining the alveoli. Phloxine-methylene blue stain.  $\times 150$ .

FIG. 2. Case 2. Right lower lobe. Perivascular infiltration of lymphocytes and plasma cells. Phloxine-methylene blue stain.  $\times 150$ .

1



2



Parker, Jolliffe, Barnes, and Finland

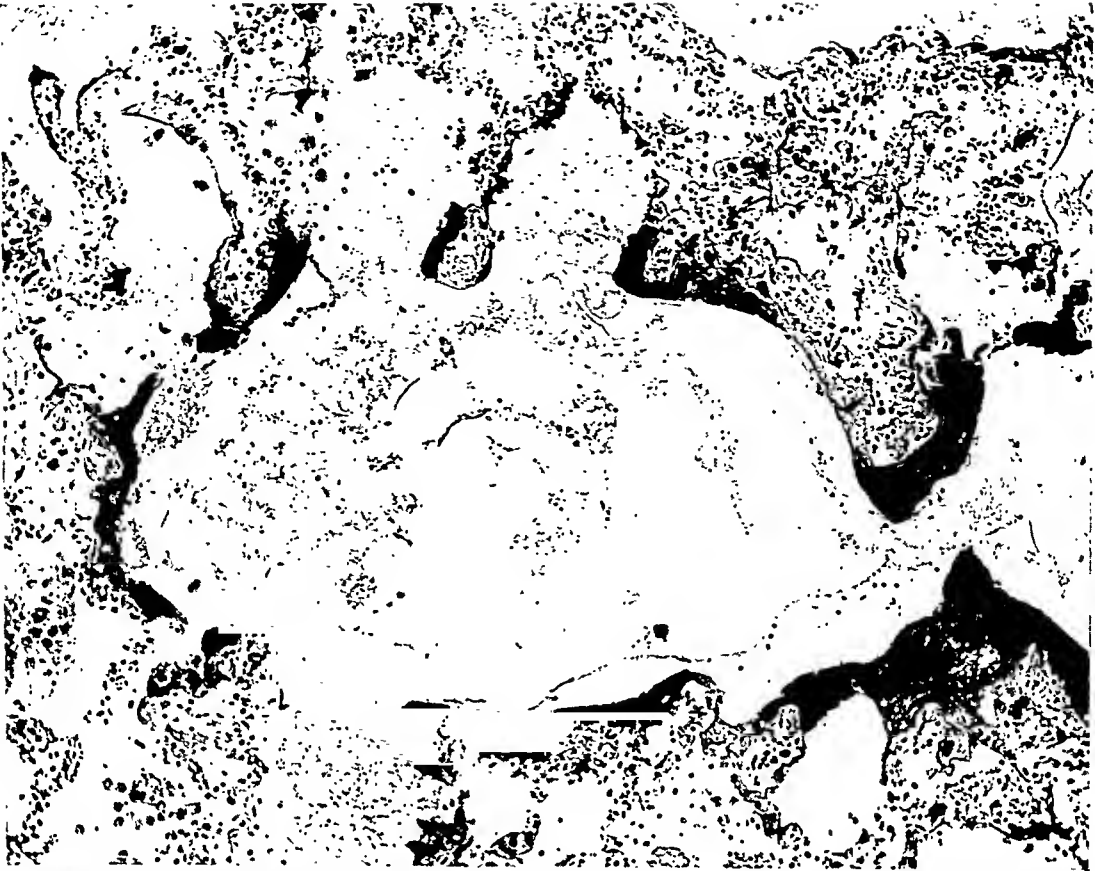
Lungs Yielding Influenza Virus

PLATE 154

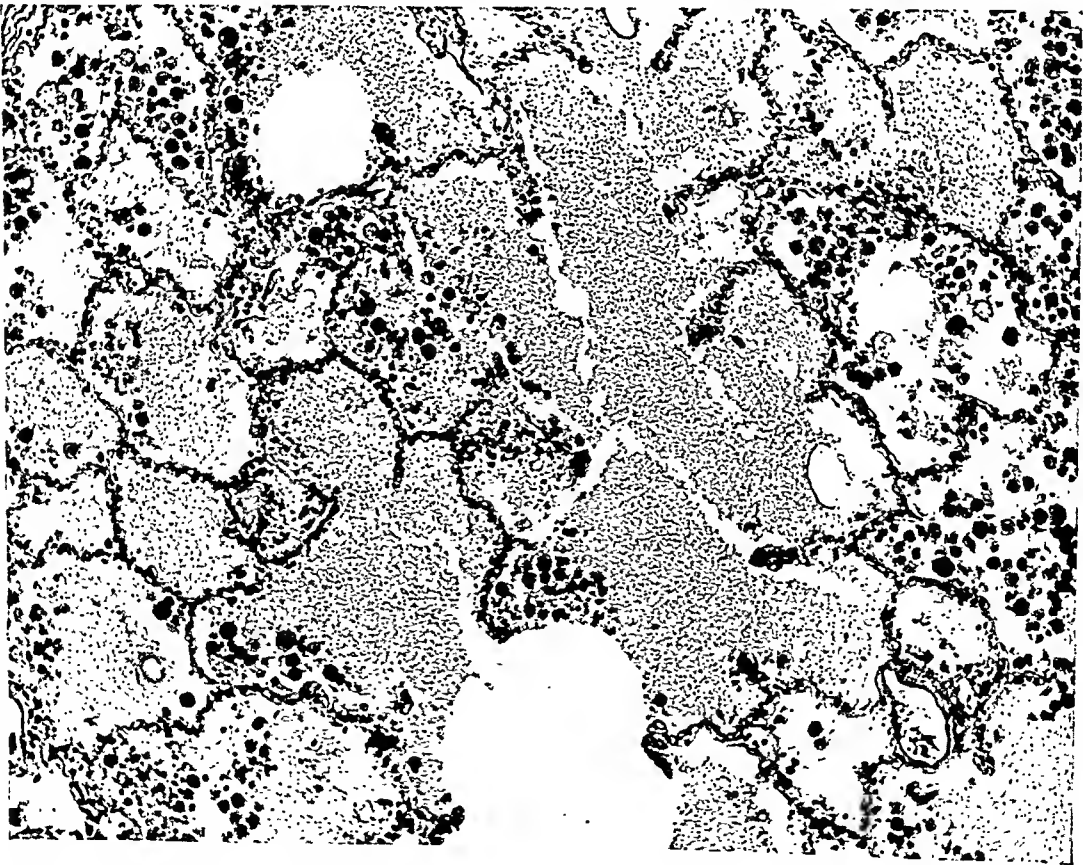
FIG. 3. Case 4. Dilated alveolar duct lined with dense, hyaline membrane.  
Phloxine-methylene blue stain.  $\times 125$ .

FIG. 4. Case 4. Alveoli show edema and an exudate of large mononuclear cells.  
Phloxine-methylene blue stain.  $\times 125$ .

3



4



Parker, Jolliffe, Barnes, and Finland

Lungs Yielding Influenza Virus

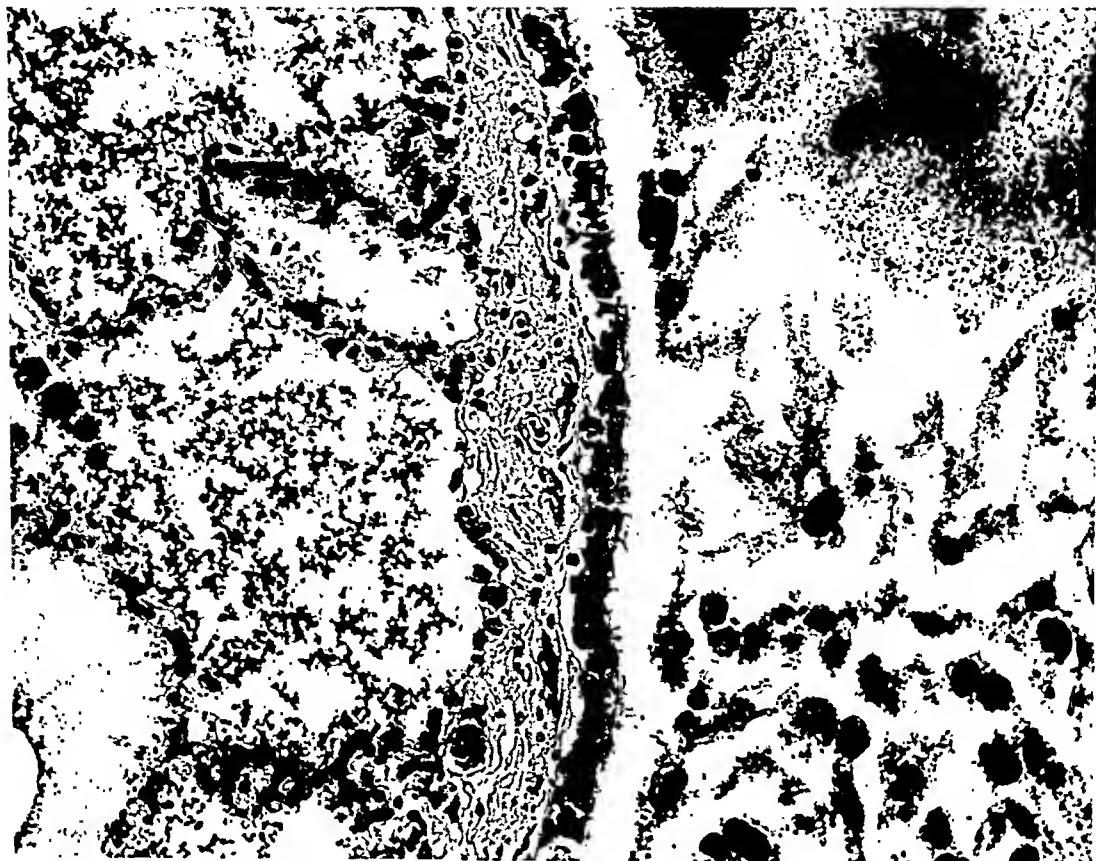


PLATE 155

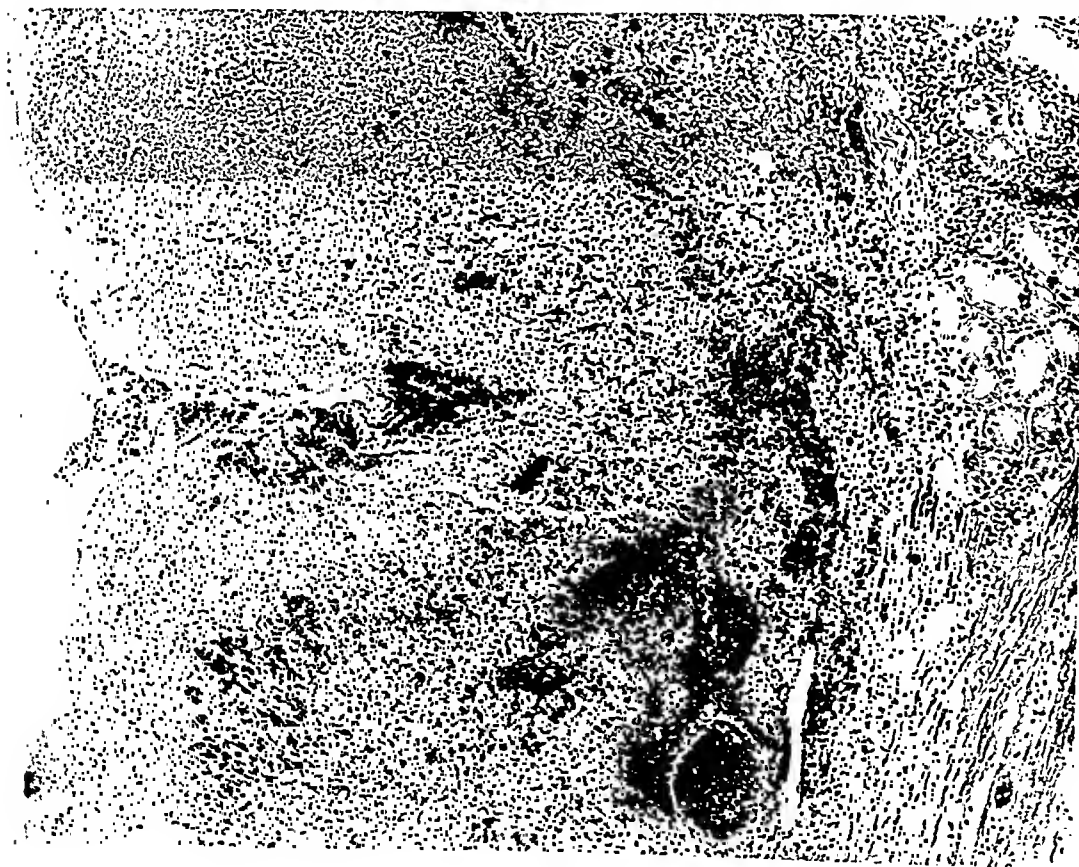
FIG. 5. Case 5. Right upper lobe. Epithelium of bronchiole is intact. Lumen contains mucus and large mononuclear cells. Alveoli show edema. Phloxine-methylene blue stain.  $\times 150$ .

FIG. 6. Case 1. Necrotizing tracheitis. Phloxine-methylene blue stain.  $\times 80$ .

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6



Parker, Jolliffe, Barnes, and Finland

Lungs Yielding Influenza Virus



# THE SIGNIFICANCE OF HYPEREMIA AROUND TUMOR IMPLANTS \*

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While using transplantable tumors in mice it was noticed that intense hyperemia develops around the tumor implants. An area of redness appears around the fragment of tumor within 18 hours after implantation, presumably before the amount of blood could be much increased by formation of new vessels.

A survey revealed a rather extensive literature upon vascular proliferation<sup>1-5</sup> in tumors, but an explanation of the hyperemia was not found. As the survival of a tumor in its host must depend in great part upon the establishment of an adequate blood supply, it is important to understand the mechanisms by which this is accomplished. Since local hyperemia is the first visible effect of the tumor upon the vessels of the host, preceding the formation of more intimate relationships between the host's vessels and the tumor, the significance of the hyperemia should be determined. It was for this purpose that the following experiments were done.

## GROSS AND MICROSCOPIC OBSERVATIONS OF THE HYPEREMIC RESPONSE

When a fragment of transplantable tumor is implanted subcutaneously in the flank of a mouse it becomes surrounded within 18 hours by a pink blush. This soon develops into a deep red zone, 1 to 3 mm. wide, around the tumor. Engorged tiny vessels form tortuous channels, interlacing to make dense meshworks in the vicinity. The vascular engorgement extends eventually to the large vessels issuing from the axilla and groin. By the third or fourth day the hyperemia is extremely intense, and, when the skin is reflected to reveal it, is so conspicuous as to be apparent to an observer several yards away. The hyperemia persists until the tumor kills the mouse.

Microscopically, dilatation of capillaries to form large channels is apparent (Fig. 1). The vessels dilate until by the third day they measure 3 or 4 times the diameter of normal vessels. These vascular changes in the tissues adjacent to the implanted tumor are apparent before the ingrowth of vessels into the tumor, or the envelopment of vessels by it.

### *1. Failure of Homologous Adult Muscle Tissue to Excite Local Hyperemia*

The first question we attempted to answer was, does the hyperemia around the tumor implants depend only upon the presence of foreign

\* Aided by a grant from The International Cancer Research Foundation.

Received for publication, July 28, 1945.

tissue? Would any piece of tissue, for example, homologous adult muscle implanted subcutaneously, cause local hyperemia?

Fresh muscle tissue from the thigh of an adult C57 mouse was implanted subcutaneously in the right flanks of 18 mice of the same strain. Into the left flanks of these mice was implanted Tumor 241\* (this tumor is a fibrosarcoma, Fig. 2, induced several years ago with dibenzanthracene and since maintained by serial transfer in this strain of mice, in which it "takes" 100 per cent). Individual mice were sacrificed from 27 hours to 8 days after implantation of the tissues, and the local vascular reaction on the two sides was compared.

Intense hyperemia was excited by Tumor 241 within 27 hours, and the hyperemia increased during the following days as the tumor grew larger. In contrast, no grossly perceptible changes were apparent in the vessels surrounding the implanted muscle fragments in any of the animals at any time (Fig. 3). The tumor implants consistently increased in size, while the pieces of muscle tissue gradually decreased in size, becoming barely perceptible by the eighth day.

From this experiment it appeared unlikely that the hyperemia resulted from the presence of foreign tissue as such.

## *II. Failure of Heterologous Tumor Tissue to Excite Hyperemia*

Could this hyperemia depend upon the *neoplastic* character of the implants? If so, any neoplastic tissue should cause this local vascular response, for example, a tumor from an alien species.

Fragments of a transplantable rat tumor† were implanted in one flank of 24 C57 mice, while mouse Tumor 241 was implanted in the other flank. Individual mice were sacrificed from 2 to 10 days after implantation, and the state of the local vascular system around the implants was observed. Tumor 241 excited the characteristic vascular response, whereas the rat tumor caused no perceptible hyperemia in any of the animals. Tumor 241 grew progressively during the 10-day period, while the rat tumor steadily regressed.

This result makes it unlikely that the hyperemia depended upon the neoplastic character of the tissue.

## *III. The Induction of Local Hyperemia by Homologous Embryonic Tissue*

Since the hyperemia did not appear to depend upon the presence of foreign tissue, nor upon the neoplastic character of the tissue, could it de-

\* The mouse tumors used in this investigation were obtained from Dr. Margaret R. Lewis, Wistar Institute, Philadelphia.

† Tumor 303, a transplantable fibrosarcoma of the rat originally induced by methylcholanthrene, obtained from Dr. E. H. Yeakel, Wistar Institute, Philadelphia.

pend upon the presence of proliferating cells? In both of the preceding experiments the test implants (adult homologous muscle and heterologous tumor) failed to grow. Would tissue, other than neoplastic tissue, which grew cause hyperemia? For example, would homologous embryonic tissue excite the hyperemia?

Embryos were removed from a mouse nearing completion of gestation. Pieces of tissue were taken from the thighs of the embryos and implanted in the flanks of 25 adult mice of the same strain. Individual mice were sacrificed and examined from 2 to 12 days thereafter. Pronounced hyperemia surrounded the embryonic tissue. This hyperemia was fully as strong (Fig. 4) as when neoplastic tissue was implanted in a susceptible mouse. The hyperemia persisted as long as the embryonic tissue continued to proliferate. Usually by the 14th day the embryonic tissue was regressing, and as regression took place the hyperemia faded.

The result of this experiment was in accord with the hypothesis that the hyperemia was dependent upon cellular proliferation.

#### *IV. Further Test of Dependence of Hyperemia on Proliferating Cells*

However, it was possible to devise a more nearly crucial experiment by taking advantage of the following fact:

Mouse Tumor 1, an induced fibrosarcoma that "takes" 100 per cent in Bagg albino mice, when implanted in C57 mice grows for a few days and then regresses, leaving the mice resistant to subsequent implants of this tumor.<sup>6</sup> If the hyperemia depended upon the presence of proliferating cells, Tumor 1 in C57 mice should produce hyperemia upon the first implantation during the short period of growth of the implants. But the same tumor in the same mice should fail to excite hyperemia in subsequent implantations, when growth would not occur because of the resistance induced by the first implants.

Pieces of Tumor 1 were implanted in the flanks of 36 C57 mice. The tumors grew slowly for a few days. Mice sacrificed and examined during the first 3 days showed strong hyperemia surrounding the tumor fragments. By the fourth day, however, the hyperemia began to fade and the tumors were regressing. Eight days after the original implantation no observable tumors were present. At this time fresh fragments of Tumor 1 were implanted in the remaining mice, and individual mice were sacrificed and examined from the third to the twelfth day thereafter. No hyperemia was seen surrounding the new implants of tumor (Fig. 5) and the tumor fragments steadily regressed.\*

Thus, the same tissue in the same mice gave different reactions de-

\* Similar observations, although as yet unpublished, were made previously by Dr. Margaret R. Lewis.

pending upon whether or not the tissue grew. This result strongly supported the concept that the hyperemia surrounding these implanted tumors was dependent upon the presence of proliferating cells.

#### *V. Failure of Artificial Hyperemia to Affect Resistance to Tumor Implants*

Since hyperemia and cellular proliferation appeared so closely inter-related, it was suggested that artificially induced hyperemia might cause a tumor to grow in a resistant host, where normally its presence would not lead to hyperemia.

To test this hypothesis advantage was taken of the fact that tumor implants grow well when placed within subcutaneous pockets in the ear of the mouse (Fig. 6). The mouse ear is also a convenient place to create hyperemia, and, further, it is possible to use one ear as a control upon the other. The pinna of the mouse is thin and the blood vessels can be observed directly both grossly and microscopically<sup>7</sup> in the living animal.

Fifteen C57 mice were made resistant to Tumor 1 by implanting it in their flanks and allowing it eventually to regress. When regression was complete, as judged by the absence of visible or palpable tumor, the left pinna of each mouse was irradiated with ultraviolet light, sufficient to produce hyperemia (6 to 7 minutes at 12 inches using a UVIARC lamp). After this irradiation, tiny pieces of Tumor 1 were implanted in both the left (irradiated) and right (nonirradiated) ears. The nonirradiated ear thus served as a control on the tumor resistance in each animal. The hyperemia in the left ear appeared on the day after irradiation and persisted for about a week, during which time the tumors were observed. No growth occurred in either ear of any animal. Thus the attempt to overcome the induced tumor resistance by artificially exciting local hyperemia around the tumor implants was unsuccessful.

From this experiment it is concluded that the failure of growth of tumor in a tumor-resistant animal depends upon other factors than the absence of hyperemia.

Hyperemia develops because the tumor grows, not vice versa.

#### DISCUSSION

The first visible effect of an implanted tumor upon the blood vessels of the host is local hyperemia. This hyperemia precedes the growth of new vessels into the tumor, or the envelopment of vessels by growth of the tumor. The experiments reported in this paper indicate that the hyperemia is excited not by neoplastic tissue as such, but by the presence of proliferating cells. This suggests the early establishment of a

reciprocal relationship between the tumor and the local vascular system of the host. If the tumor cells proliferate, hyperemia is excited, increasing the flow of blood to the part. The increased flux of blood would presumably operate advantageously to the mass of actively dividing cells. Hyperemia is the first apparent step in the process whereby the host's vessels and the growing tumor become intimately associated. Whether vascular proliferation is aided by, or dependent upon, a preceding hyperemia has not been determined.

### SUMMARY AND CONCLUSIONS

Intense local hyperemia is a constant finding in the vicinity of transplantable mouse tumors. Experiments were directed toward determining the significance of this phenomenon.

It was found that hyperemia appeared within 18 hours after implantation and was progressive thereafter so long as the tumor grew.

Implants of homologous adult muscle tissue failed to produce hyperemia, indicating that hyperemia was not caused merely by the presence of foreign tissue.

Heterologous tumor implants did not produce hyperemia, showing that the vascular response did not depend upon the fact that the tissue was neoplastic.

Homologous embryonic tissue, which grew for a time in the host, excited strong hyperemia that faded as the embryonic tissue finally regressed. This suggested that the hyperemia was due to the presence of proliferating cells.

Tumor 1, from Bagg albino mice, when implanted in C57 mice grows for a time and then regresses, leaving the mice resistant to subsequent implants of this tumor. It was found that the initial implants of Tumor 1, during their short growth period, produced hyperemia, whereas subsequent implants of the same tumor in the same mice did not grow and did not cause hyperemia.

It is concluded from the several experiments that the hyperemia around transplanted mouse tumors is due to the presence of proliferating cells.

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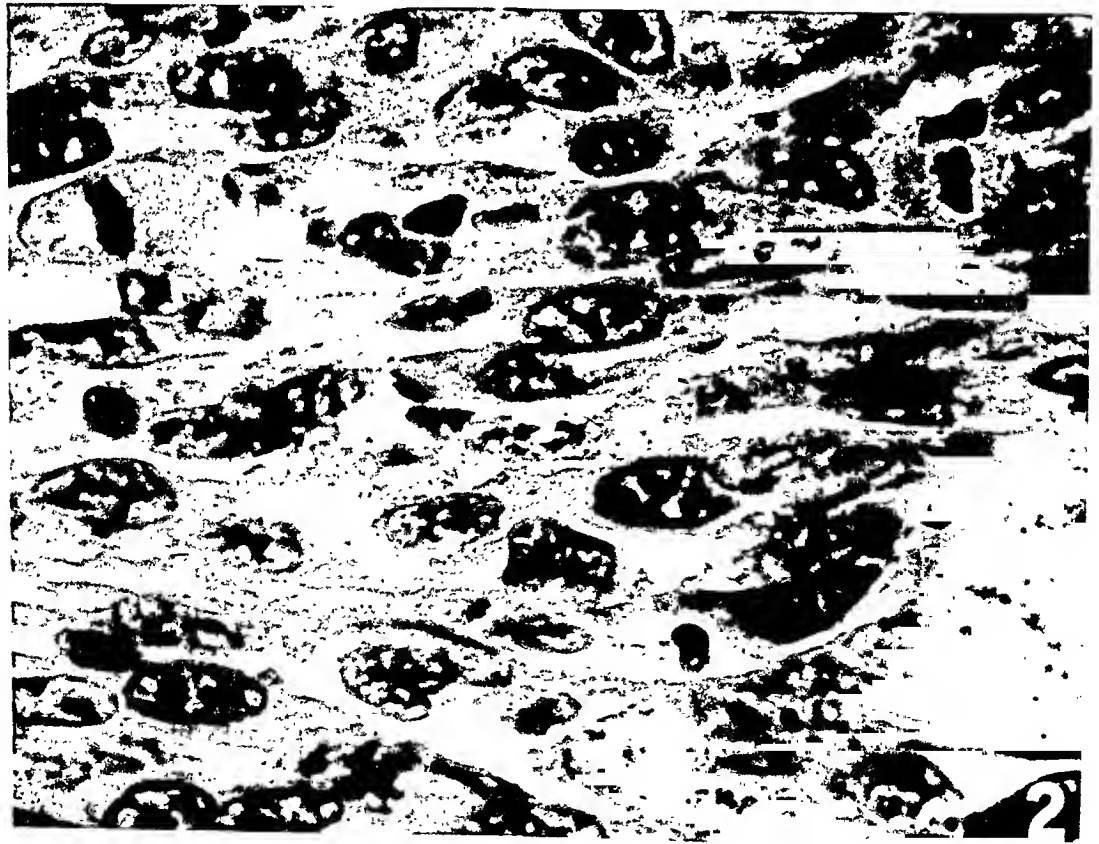
## DESCRIPTION OF PLATES

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### PLATE 156

FIG. 1. Low-power photomicrograph of a transplantable mouse tumor 5 days after subcutaneous implantation. The tumor is in the right lower corner. Several large blood vessels are seen at the edge of the growing tumor.  $\times 110$ .

FIG. 2. High-power photomicrograph of a transplantable mouse tumor (Tumor 241) used in the experiments described in this paper. Structure is that of a fibrosarcoma.  $\times 970$ .



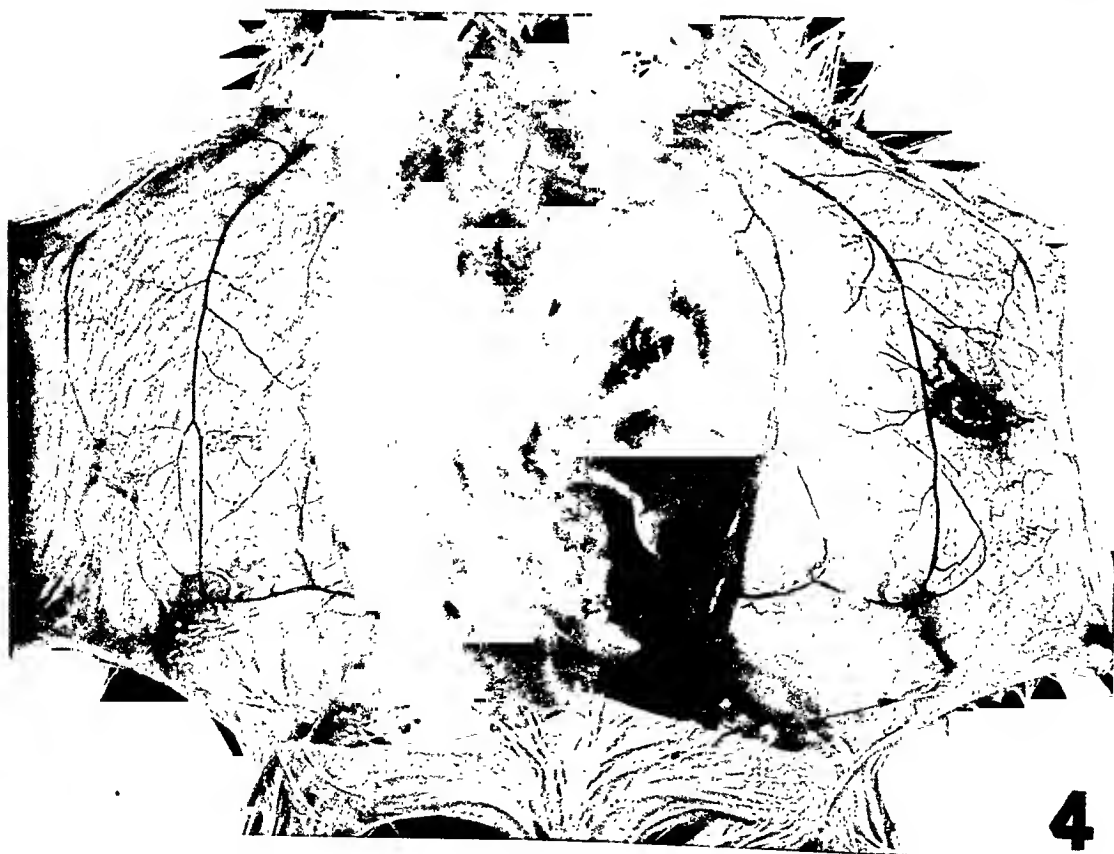
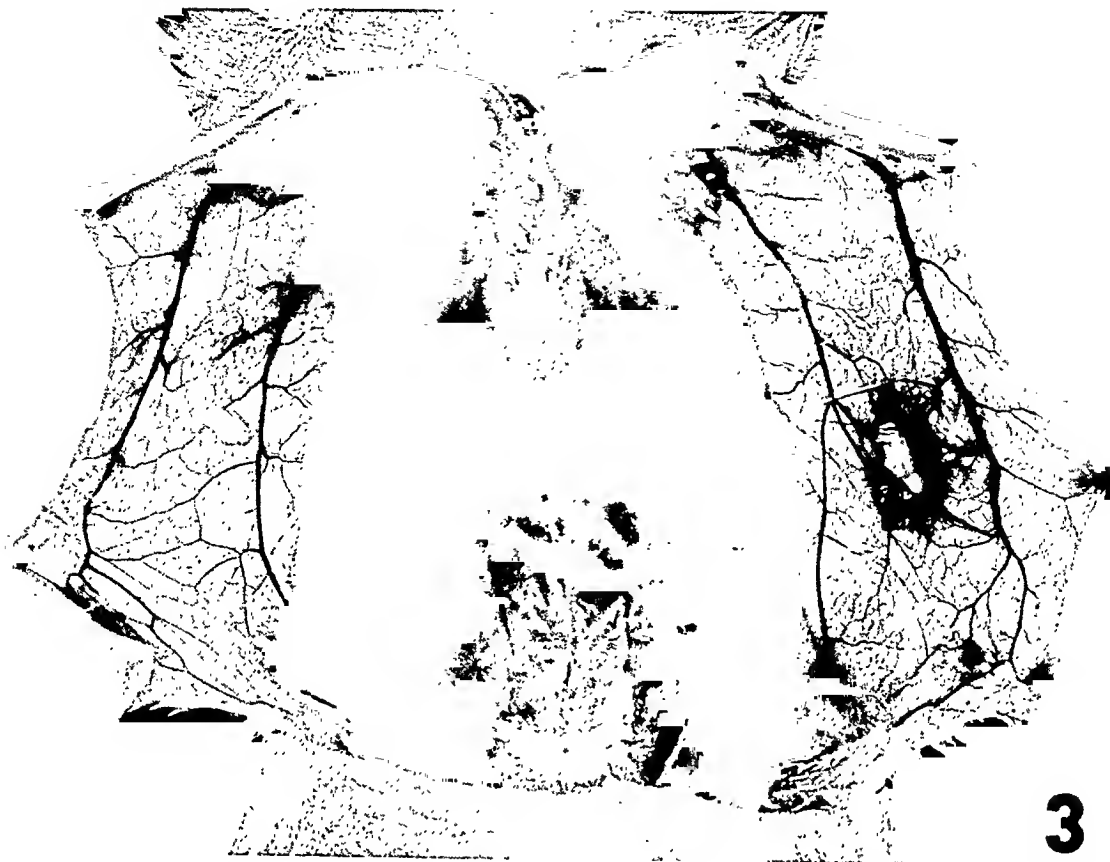
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Hyperemia Around Tumor Implants

PLATE 157

FIG. 3. A mouse, with the skin reflected to expose subcutaneous implants and vessels in flanks. The fragment on the right (in the photograph) is transplantable tumor, 3 days after implantation. Of note are the intense hyperemia and the dilatation of even the large vessels. The fragment on the left is homologous muscle tissue. Hyperemia is not present around the muscle.

FIG. 4. Implants of homologous embryonic tissue in the right (in the photograph), and homologous adult muscle in the left, flanks of a mouse, 5 days after implantation. The embryonic tissue has excited a strong local hyperemia. No hyperemia surrounds the fragment of adult muscle tissue, which is barely discernible just medial to the largest vessel, near its mid-point.



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PLATE 158

FIG. 5. Tumor implants in resistant host. A C57 mouse in which a fragment of Tumor I (from a Bagg albino mouse) was previously allowed to grow and regress, thus establishing resistance to further implants of Tumor I. Subsequently Tumor I was implanted (pale linear mass, right side of photograph) and failed to excite hyperemia. In contrast, Tumor 241, to which the animal was susceptible, excited intense local hyperemia as seen on the left. The implants are of 3 days' duration.

FIG. 6. Photograph of mouse showing use of the pinna as a site of tumor implantation. The vessels are widely dilated in the tumor-bearing ear.



Coman and Sheldon

Hyperemia Around Tumor Implants



# MEDIASTINAL CHORIONEPITHELIOMA IN A MALE

## A CASE REPORT \*

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The knowledge that chorionepitheliomas in females originate in the epithelium of the chorionic villi was first established by Marchand<sup>1</sup> in 1895, at which time he carefully elaborated and clarified the histology of this tumor. It was not until 1902 that Schlagenhauser,<sup>2</sup> among others, described the development of this tumor in males from chorion-epithelium arising from germinal cells of the testis or teratomatous tissue. Since that time, despite the many reports of chorionepitheliomas of genital origin in males, very few extratesticular tumors of this type have been reported. Moreover, the lack of adequate criteria for the establishment of the extragenital origin of these neoplasms has caused considerable controversy among the writers on this subject as to just which cases may be considered sufficiently well documented to be accepted as extragenital tumors. Heaney,<sup>3</sup> Kantrowitz,<sup>4</sup> Erdmann, Brown, and Shaw,<sup>5</sup> and others have each published collected series in which the cases accepted differ from author to author.

Much of the confusion has arisen from the fact that tumors primary in the testes may not only be extremely small, but moreover may spontaneously regress and "heal," leaving microscopic scarring as the only permanent vestige of the former neoplasm (Prym,<sup>6</sup> Craver and Stewart<sup>7</sup>). Therefore, before a case can be definitely accepted as of extragenital origin, the testes must be ruled out as the primary site. Cases in which markedly atrophic testes have been found, or in which no microscopic examination of the genitalia was done, must be excluded. Whether serial sections of the genitalia must be examined in all cases, as suggested by Prym and by Erdmann, Brown, and Shaw, would seem to depend largely upon the individual case, and we agree with Frank<sup>8</sup> that a distinct mediastinal teratoma is a reasonable site of origin for a mediastinal chorionepithelioma when the testes have been carefully studied by multiple sections and found to be negative, even if not by serial sections.

On the basis of these criteria, 14 cases may be listed as established extragenital chorionepitheliomas in males, reported to date (Table I).

The purpose of this report is to present an additional case, primary in the mediastinum, occurring in a 26-year-old male who entered the Boston City Hospital in 1935 and was autopsied at the Mallory In-

\* Received for publication, September 20, 1945.

† Now on leave of absence with the U. S. Navy.



stitute of Pathology in the same year. The extragenital origin of this tumor has been adequately established by careful examination of the testes, which were not only grossly negative on serial section but were likewise free of neoplasm in the thirty-two representative blocks of the testes studied microscopically.

While no distinct, well differentiated teratoma could be identified within the mediastinal tumor, the considerable size of the tumor (10 by 9 by 8 cm.) made serial microscopic sections of the mass impractical. However, the identification of two distinct histologic types of tumor

TABLE I  
*Reported Cases of Extragenital Chorionepitheliomas in Males*

Case	Author	Date	Age	Primary site
1	Miller and Browne <sup>9</sup>	1922	39	"Posterior to liver"
2	Krasnjanskaja <sup>10</sup>	1930	72	Undetermined, "not in testes"
3	Arendt <sup>11</sup>	1931	20	Mediastinum
4	Heaney <sup>3</sup>	1933	40	Retroperitoneal
5	Kantrowitz <sup>4</sup>	1934	22	Anterior mediastinum
6	Fenster <sup>12</sup>	1934	27	Retroperitoneal
7	Gerber <sup>13</sup>	1935	23	Retroperitoneal
8	Weinberg <sup>14</sup>	1939	70	Bladder
9	Mathieu and Robertson <sup>15</sup>	1939	27	Retroperitoneal
10	Erdmann, Brown, and Shaw <sup>5</sup>	1941	45	Retroperitoneal
11	Hyman and Leiter <sup>16</sup>	1943	57	Bladder
12	Plenge <sup>17</sup>	1944	30	Retroperitoneal
13	Stowell, Sachs, and Russell <sup>18</sup>	1945	15	Intracranial
14	Laipply and Shipley <sup>19</sup>	1945	13	Mediastinal

within the thoracic growth, namely, chorionepithelioma and embryonal carcinoma, makes the teratomatous origin of this mixed neoplasm seem likely.

#### REPORT OF CASE

E. C. H., a 26-year-old elevator operator, was admitted to the Boston City Hospital on March 28, 1935, complaining of pain in the right chest. He had been perfectly well until 3 weeks before, at which time he had experienced a heavy feeling under the sternum while at work. He also began at that time to feel weak and to lose his appetite. The chest symptoms soon developed into sharp, knife-like pain radiating from the sternum over the right upper chest towards the shoulder, and also from the region of the right nipple over the shoulder and down to the right mid-back. A cough appeared which was at first productive of brownish yellow sputum, which later was tinged with bright red blood. The pain soon became severe enough to interfere with his sleep, causing him to sit up in bed in a vain effort to gain relief. Coughing or deep breathing aggravated the pain, and approximately 1 week after the onset of his first symptoms he noted marked shortness of breath both on exertion and at rest. He had five or six attacks of epistaxis. Later he began to feel nauseated and vomited twice. The vomitus was not bloody. He stopped work the day before admission because of the pain and the increasing dyspnea, weakness, and dizziness. He stated that he believed he had lost 18 pounds during the 2 months preceding admission.

The past history and family history were irrelevant.

*Physical Examination.* The patient was a well developed and well nourished adult male who was lying in bed and appeared ill and uncomfortable. His voice sounds were normal but he talked in paroxysms due to the dyspnea created by the effort of talking.

The eyes, ears, nose, and throat were normal.

The neck showed distention of the superficial veins with marked tortuosity. A tracheal tug was palpable above the manubrium. The thorax appeared slightly asymmetrical with a suggestive prominence of the right chest, which appeared to move less than the left on respiration. Both breasts were more prominent than is normal, with deep, circular, firm areas about 4 to 5 cm. in diameter under each nipple. These masses were freely movable and nontender. There was dullness to flatness over the upper right lung field and breath sounds were absent in this area. The lung base on this side was high and there was hyperresonance over the right lower chest. The left lung and the heart were within normal limits.

The external genitalia were normally developed. Both testes were regular, equal in size, and of normal elasticity.

Axillary and inguinal lymph nodes were bilaterally palpable but not markedly enlarged.

The remainder of the physical examination was negative.

*Laboratory examinations* revealed the following: red blood cell count, 4,400,000 cells per cmm.; hemoglobin, 74 per cent; white blood cell count, 6,500 cells per cmm.; differential, normal except for 5 per cent eosinophils. The urine contained no albumin, sugar, or abnormal sediment.

*Clinical Diagnoses.* Occlusion of the superior vena cava; right hydrothorax; gynecomastia; lymphosarcoma of mediastinum; question of teratoma of mediastinum.

*Clinical Course.* The patient went progressively downhill with rapidly increasing dyspnea and cyanosis. The cough became more severe and hemoptysis more marked. On the eleventh day of his hospital stay death occurred suddenly without premonitory signs or symptoms.

#### POST-MORTEM EXAMINATION

Autopsy was performed 12 hours after death. The body was that of a well developed and well nourished, young adult male. Both breasts were hypertrophied and firm. Cross section revealed each to be composed of a circular, flat mass of white, firm tissue, 4 cm. in diameter and 2.5 cm. thick, centered under the nipple.

The peritoneal cavity was without pathologic change.

The pleural cavity on the right contained 900 cc. of dark red, cloudy fluid. The left cavity contained approximately 100 cc. of similar fluid. There was a large, moderately firm, friable, dark red mass with yellow-white and tan areas in it, measuring 10 by 9 by 8 cm., in the superior portion of the mediastinum. It was attached firmly to the upper lobe of the right lung medially and to the anteromedian surface of the left upper lobe by a few fibrous adhesions. The anterior portion of the great vessels and trachea and bronchi were embedded in the mass which on section proved to be friable, spongy, red-brown tissue similar to that described above. Postero-inferiorly the neoplastic tissue ap-

peared firmer and yellow-white. Invasion of the anterior wall of the superior vena cava at the junction of the left and right innominate veins by neoplastic tissue had occurred with the production of almost complete occlusion of the superior vena cava. The tumor caused a marked compression of the main portion of the azygos vein, as well as marked compression with almost complete occlusion of the branches of the right bronchus entering the upper and middle lobes. A friable, red, spongy mass, in which there were large areas of blood clot, replaced the normal structure of the right upper lobe and upper half of the middle lobe. This mass was slightly larger than the mediastinal tumor and was firmly fixed to the parietal pleura by fibrous adhesions.

The pericardial cavity contained 100 cc. of blood-tinged fluid. A friable, red tumor nodule extended through the anterosuperior portion of the parietal pericardium and protruded into the pericardial sac for 2 to 6 mm. over an area 3 by 1 cm. The tumor nodule was not adherent to the epicardium. The heart weighed 340 gm. and was entirely within normal limits.

The left lung weighed 750 gm. The right was not weighed. In the right upper lobe and upper half of the middle lobe was found the mass previously described. The cut surface of the tissue exactly resembled the tumor previously described, and serial sections of the mass showed it to be continuous with the tumor in the mediastinum. The remainder of the middle lobe and the lower lobe were flabby, collapsed, and subcrepitant with many round metastatic nodules, varying from 0.5 to 3.5 cm. in diameter, scattered throughout. The left lung contained nodules similar both in size and character, scattered throughout a gray-red, crepitant parenchyma.

The liver weighed 2230 gm. In the anterosuperior portion of the right lobe there was an irregularly spherical mass of friable, spongy, dark red tumor, 8 cm. in diameter, similar to that found in the lung and mediastinum. This had extended through the capsule and was attached to the surface of the right dome of the diaphragm. The remainder of the liver was brown-red and was of the usual consistency.

The brain weighed 1440 gm. There was a moderate generalized flattening of the convolutions. A mass of obvious neoplastic tissue, 2 cm. in diameter, was found in the right cerebellar hemisphere involving the medio-inferior portion of the dentate nucleus and bulging into the fourth ventricle. The pituitary gland was grossly negative.

The remainder of the post-mortem examination was negative with particular attention having been devoted to the genital organs (penis, prostate, seminal vesicles, and testes).

### *Microscopic Examination*

The only histologic features of interest in addition to the tumor were found in the breasts, pituitary gland, and testes. In the soft, hemorrhagic areas the mediastinal tumor presented the typical microscopic picture of a chorionepithelioma with masses of anaplastic syncytium-like cells in a bloody matrix (Fig. 1). The cells were characteristically extremely pleomorphic, with acidophilic cytoplasm, and sometimes with indistinct cell boundaries forming syncytium-like masses. Mitotic figures were numerous. In contrast, sections from the more solid, white areas of the tumor showed a uniform growth of large, round, undifferentiated tumor cells with little cytoplasm and regular, round nuclei having finely divided chromatin and widely scattered mitotic figures (Fig. 2). Careful search for well differentiated, teratomatous tissue was unsuccessful. However, as was previously stated, the occurrence of two distinct histologic types of neoplasm within a single tumor mass strongly suggested the differentiation of a teratoma into two related neoplasms.

The breasts showed fairly generalized cellular hyperplasia with proliferation of the duct epithelium so that the cells were heaped upon one another, creating a lining two or three cells thick (Fig. 3). There was scattered rudimentary gland production, but no evidence of secretory vacuolization of the epithelial cells could be found.

Careful microscopic search of thirty-two sections of testes and other sections of epididymides and spermatic cords failed to disclose evidence of neoplasia or residual scarring.

The tubular epithelium showed good spermatogenesis with occasional atrophic, slightly fibrosed tubules. There was a slight focal increase in the number of interstitial cells.

The pituitary body was quite remarkable. In addition to oxyphilic, chromophobic, and basophilic cells, other cells were present. The cytoplasm of these cells in hematoxylin and eosin stains was red, but not as strikingly red as the cytoplasm of the eosinophilic cells, and their contours were not distinct. These cells constituted a considerable proportion of the anterior lobe, giving the impression of hyperplasia (Fig. 4). Many mitotic figures were found.

*Anatomic Diagnoses.* (1) Chorionepithelioma and embryonal carcinoma, primary in the mediastinum and probably of teratomatous origin, with direct extension into the right lung, pericardium, and superior vena cava; (2) subtotal occlusion of the superior vena cava; (3) metastasis to both lungs, liver, and right cerebellar hemisphere; (4) compression of the azygos vein and main bronchi to the upper

and middle lobes of the right lung; (5) gynecomastia; (6) changes in the pituitary body consistent with pregnancy.

Many hormone assays were performed in this case, one on the urine prior to death and several on the urine and various tissues obtained at autopsy. The results are tabulated in Table II.

### DISCUSSION

The question whether this mediastinal tumor arose directly from aberrant sex cells in the mediastinum or through the development of a teratoma is largely academic and, for reasons previously stated, must remain in this case unanswered. According to Ewing,<sup>20</sup> these sex cells may occur anywhere along the entire length of the embryonal entoderm

TABLE II  
*Hormone Assays*

Source	Follicle-stimulating hormone	Luteinizing hormone	Estrogens
	( <i>rat units</i> )	( <i>rat units</i> )	( <i>castrate mouse units</i> )
Urine, 1 week prior to death	330,000*	50,000*	783*
Urine, autopsy	1,300,000*	330,000*	
Tumor, 1 kg. of wet tissue	1,040,000†	260,000†	60-430†
Breast, 1 kg. of wet tissue	330,000†	26,000†	less than 50†

\* Units per liter.

† Units per kg. of wet tissue.

and may thus be found within the mediastinum. However, most authors agree with Kaufmann<sup>21</sup> that true extragenital chorionepithelioma probably always arises in teratomatous tissue.

The case reported here presents a fairly typical clinical picture of mediastinal chorionepithelioma. The age of our patient was 26 years, which is 10 years less than the average age at death of the tabulated cases. Hörnicke<sup>22</sup> collected 35 cases of chorionepithelioma in males in which the age had been mentioned and 23 of the patients were between 20 to 40 years of age at death. We, too, have found that of 26 cases mentioned or reviewed in this paper, all were between 20 to 40 years of age except 6 (13, 15, 45, 57, 70, and 72 years, respectively).

The symptoms presented by our patient were characteristic of this type of mediastinal lesion, with pain referred to the anterior mid-chest and right nipple region, radiating to the shoulder and back. The intensification of the pain on coughing is common to many. The dyspnea, orthopnea, cough, bloody sputum, and weight loss presented by this

patient are likewise frequently seen in these cases. On occasion, cerebral symptoms are encountered, usually due to metastases.

Gynecomastia is a common finding and is so characteristic as to suggest the diagnosis in certain instances. The chorionic tissue in the tumor functions in a fashion analogous to a pregnancy with the production of (1) follicle-stimulating and luteinizing hormones that can be demonstrated in the urine and blood, (2) changes in the pituitary glands resembling those of pregnancy, and (3) breast changes. In eight instances in the literature colostrum formation was noted in these cases of gynecomastia. This finding is not common, however, and the absence of colostrum formation in this case, as well as the rarity of its

TABLE III

*Time Relationships of the Breast Changes Associated with Chorionepithelioma in Males*

Authors	Age	Duration	Breasts
Entwisle and Hepp <sup>23</sup>	22 years	5 months	Gynecomastia with colostrum
Friedländer and Moses <sup>24</sup>	38 years	6 months	Gynecomastia with colostrum
Melicow <sup>25</sup>	20 years	7 months	Gynecomastia
Jüngling <sup>26</sup>	27 years	6 months	Gynecomastia
Bonn and Evans <sup>27</sup>	34 years	5 months	Gynecomastia
Gilbert <sup>28</sup>	25 years	3 months	Gynecomastia and microscopic colostrum
MacDonald <sup>29</sup>	40 years	3 months	Gynecomastia
Kirwin <sup>30</sup>	34 years	11 weeks	No gynecomastia
Jüngling <sup>26</sup>	26 years	8½ weeks	Gynecomastia not mentioned clinically, found histologically
Fortner and Owen, quoted by Mathieu and Robertson <sup>15</sup>	38 years	2 months	Marked hypertrophy
	40 years	1 month	Slight hypertrophy

occurrence in previously described cases, is probably due to the fact that the undeveloped male breast requires a longer time to develop glandular hyperplasia and secretory activity than does the female breast. Our patient died 1 month after the onset of his clinical symptoms and probably there was insufficient time to permit the development of significant glandular secretory activity.

Analysis of the cases in the literature in which the condition of the breasts was mentioned (Table III) would seem to indicate that at least 3 months must elapse before colostrum formation can be reasonably anticipated, and even then its occurrence is not invariable.

The widespread assumption that gynecomastia is preceded by atrophy or destruction of testicular tissue would appear to be disproved by this case of extragenital chorionepithelioma as well as by many reported cases, in all of which intact testes were found.

The development of so-called "pregnancy cells" in the pituitary

body has long stimulated observers to speculate as to their significance. Erdheim,<sup>31</sup> in 1936, expressed the hypothesis that the cells might be endocrinologically active, secreting a growth-promoting hormone. Cases in which chorionepithelioma have been found in children tend to support this view, such as the series reported by Sturley,<sup>32</sup> in 1942, of thirteen cases of ovarian chorionepithelioma in children up to the age of 13 years with the finding of precocious sexual development in all. According to Erdheim, many of the authors' preoccupation with the abnormal sexual development in children frequently led to their overlooking a markedly precocious bodily development. Fasold<sup>33</sup> and Siegmund,<sup>34</sup> both reporting well documented cases of chorionepithelioma in childhood, noted marked growth in height, disproportionate for their age, in children with these tumors, possibly associated with the pregnancy-like cells in the pituitary body.

Ever since the time of Meyer<sup>35</sup> and Aschheim and Zondek,<sup>36</sup> the Aschheim-Zondek test has been used to detect chorionepithelioma in females. Heidrich, Fels, and Mathias<sup>37</sup> made the analogous observation in males, and the test has since been used in males and females not only to confirm the diagnosis, where suspected, but also to detect recurrence or metastases of the tumor postoperatively by demonstrating in the urine hormone titers in excess of the levels encountered in normal pregnancies, with progressively rising values as the tumor increases in size.

#### SUMMARY AND CONCLUSIONS

1. A case of a 26-year old male with a large chorionepithelioma primary in the mediastinum, presumably arising in a teratoma, is described.
2. Serial sections of both testes taken 2 mm. apart showed no gross or microscopic evidence of tumor.
3. Gynecomastia, a positive Aschheim-Zondek test, and changes in the pituitary body resembling those seen in pregnancy were all present.
4. Fourteen other cases of extragenital chorionepithelioma in males have been collected from the literature.
5. The greatest incidence of these lesions is found in the third and fourth decades of life.
6. Gynecomastia, or at least glandular hypertrophy, is frequently found in these cases in males, and in certain instances colostrum formation has been observed.
7. Inasmuch as a primary tumor in the testes may be extremely small or may regress to microscopic size, metastasis from the testes must be considered and ruled out in all instances of supposed primary

extragenital chorioneplithelioma by a thorough examination of those organs, preferably by multiple sections examined microscopically.

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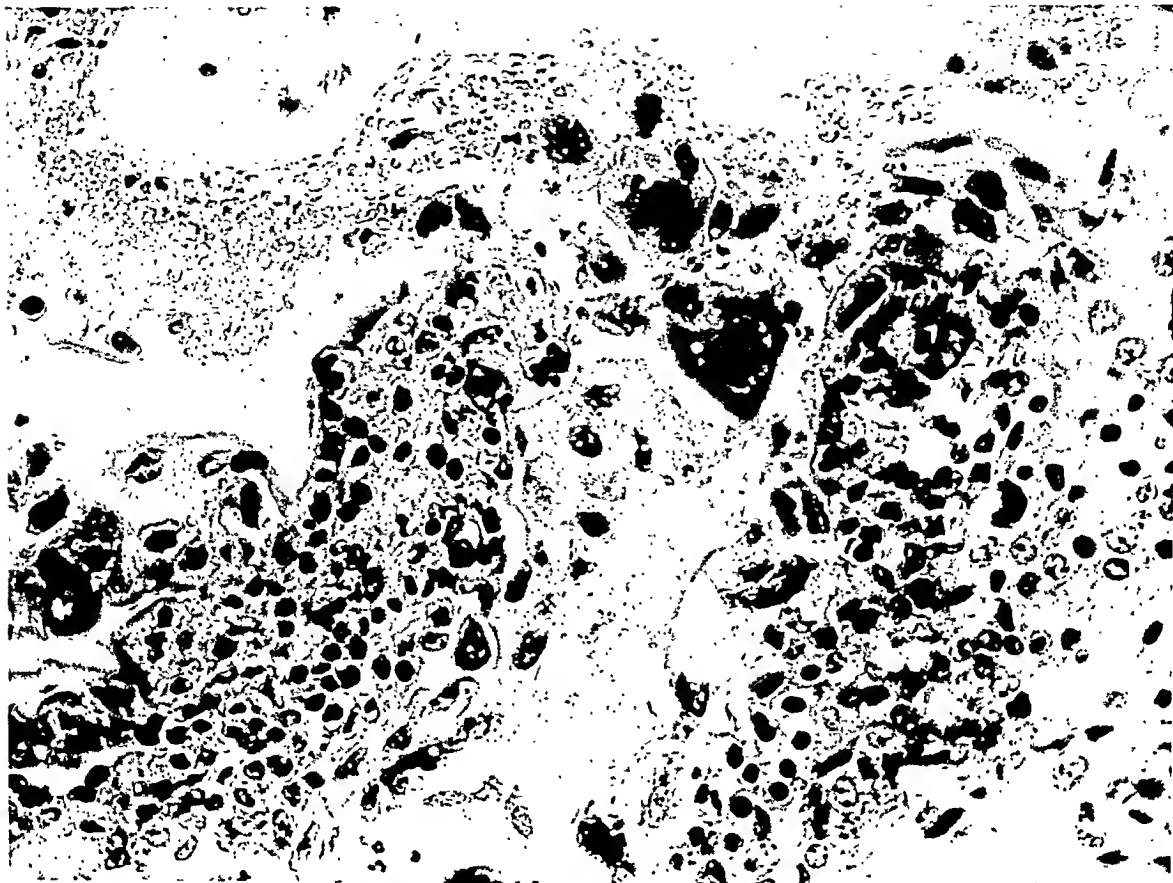
## DESCRIPTION OF PLATES

### PLATE 159

FIG. 1. Mediastinal tumor showing the two types of epithelium characteristic of chorionepithelioma. Phloxine-methylene blue stain.  $\times 175$ .

FIG. 2. Mediastinal tumor. Undifferentiated portion growing as round to polygonal cells. Phloxine-methylene blue stain.  $\times 265$ .

1



2

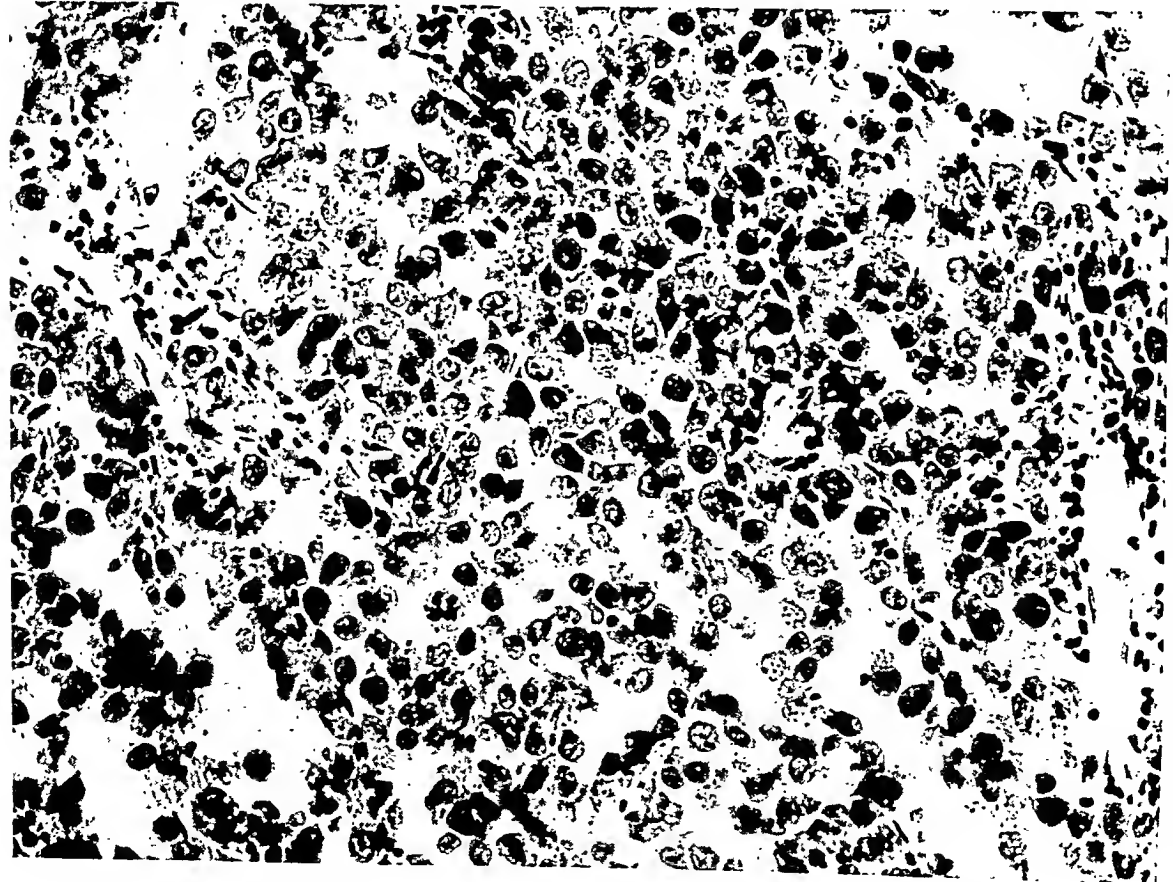
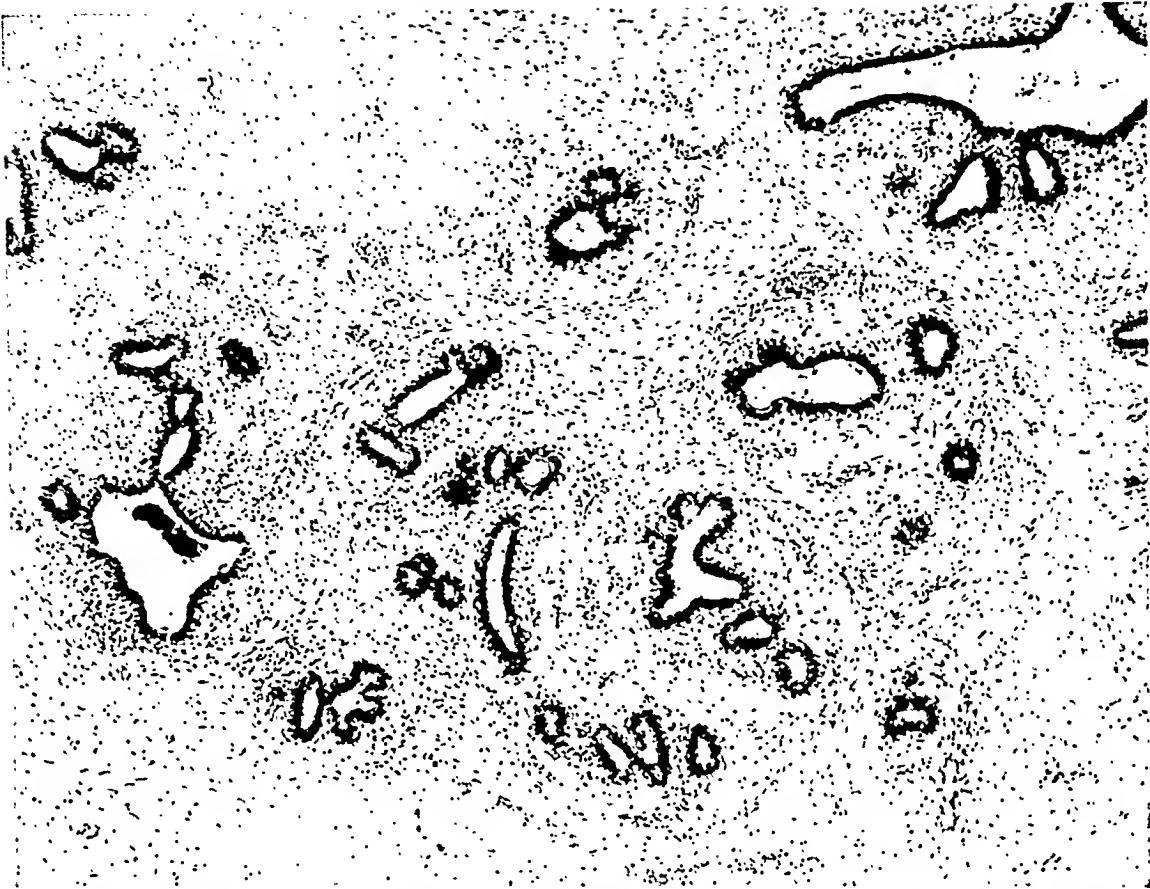


PLATE 160

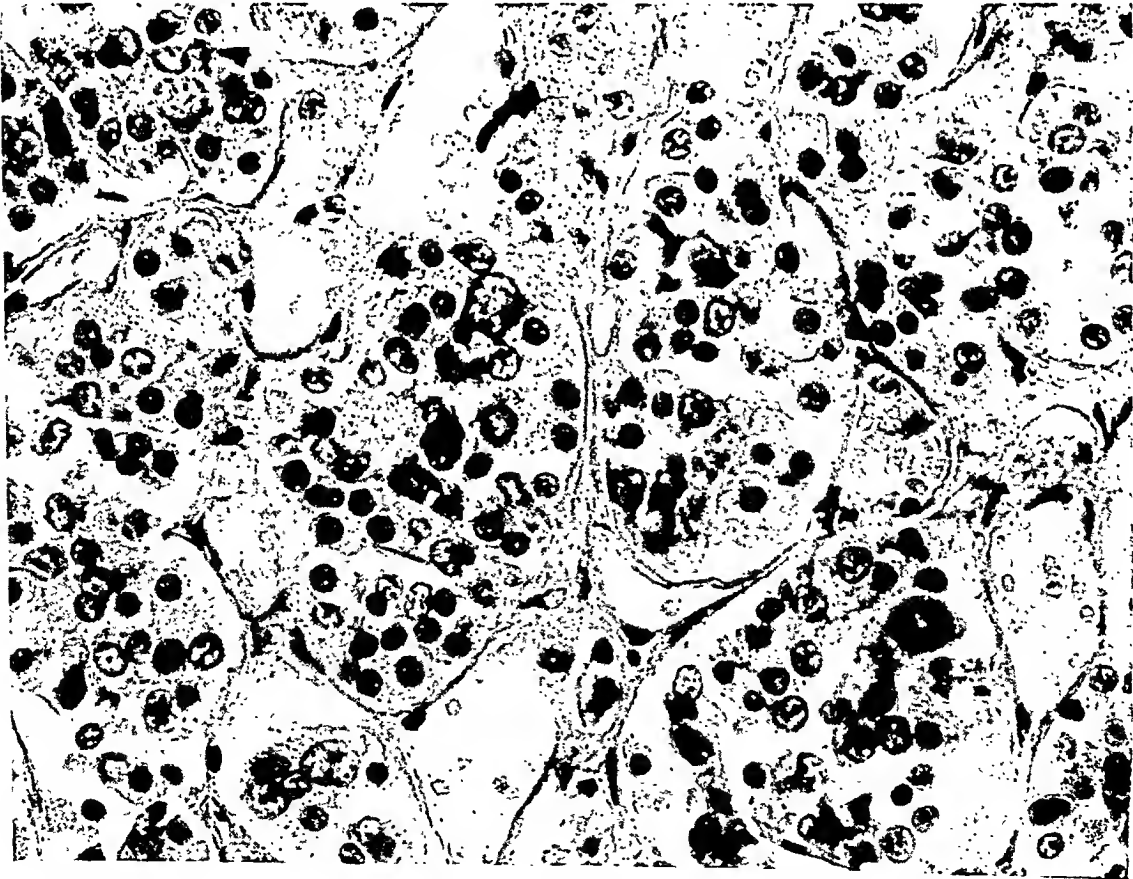
FIG. 3. Breast, showing histologic picture of gynecomastia. Phloxine-methylene blue stain.  $\times 130$ .

FIG. 4. Pituitary body. The majority of the cells are the so-called pregnancy cells. Hematoxylin and eosin stain.  $\times 575$ .

3



4





## MEDIAL HYPERPLASIA IN PULMONARY ARTERIES OF CATS \*

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In the course of pharmacologic investigation, one of us (W. M.) has examined microscopic sections from the lungs of more than 150 cats. In two of these there was advanced hypertrophy and hyperplasia of the smooth muscle of the intrapulmonary arteries, but there was nothing similar to this in the other cats examined. A third cat (no. 2), received from another source, showed the same change. We have found no reference to this lesion in cats except for notes by Ettinger<sup>1, 2</sup> on an apparently similar condition. In view of the rarity of this condition, the findings in these three cats will be described, with consideration of a number of controls.

### *Cat 1*

An adult male cat of unknown age, that weighed 3.32 kg., died 5 minutes after injection of 120 mg. per kg. of silicic acid into a saphenous vein. An incomplete autopsy was performed, and no lesions were noted except in the lungs. The lungs showed the congestion frequently associated with this experimental procedure. When parts of the lungs were preserved in a 4 per cent solution of formaldehyde, gray nodules about 2 mm. across were seen, both around the bronchi and under the pleura.

Microscopically, there were smaller separate nodules and confluent groups of similar structure, both found to represent arteries with enormously thickened walls and very small or even apparently obliterated lumina (Figs. 1 and 2). The hypertrophy was limited to the media. The endothelium, subendothelial connective tissue, and peripheral connective tissue were intact. The internal elastic lamina was moderately wavy in many instances, but otherwise not unusual. The media was formed of a very thick layer of concentrically arranged, spindle-shaped cells, with the structure of large smooth muscle cells. They were purple with Masson's and with Mallory's connective tissue stains. All of the arteries in the sections showed the same change. The lesion was clearly a chronic process, entirely unrelated to the experimental procedure. Congestion of the intra-alveolar blood vessels was attributed to the intravenous congestion. The bronchi were normal except for narrowing due to pressure from the adjacent hypertrophic arteries.

The arteries present in the sections have been measured with a

\* Received for publication, July 20, 1945.

screw micrometer in the ocular of a microscope. In many cases the arteries were cut so obliquely that accurate measurement was impossible. In others, the vessels could be measured with reasonable accuracy. The wall of each vessel (W) was measured twice in the center of each quadrant, and these eight readings averaged. The lumina (L) were measured twice in each of two axes, perpendicular to one another, and these four readings averaged. The resulting ratios (W:L) are expressed in the same terms as were used for measuring human arterioles by Kernohan, Anderson, and Keith<sup>3</sup> and Morlock.<sup>4</sup> It should be noted that the average thickness of one wall is compared to the average diameter of the lumen. The external diameter of each artery would be represented by  $2W + L$ , not by  $W + L$ . The results have been plotted in Text-Figure 1, the ordinates indicating the external diameter of the vessels, the length of the horizontal lines denoting the ratio of the average thickness of the wall of each artery to the size of its lumen (W:L). In the ten vessels measured, the average of the ratio W:L was 1.36 (eight times that of the control animals).

#### *Cat 2*

A common male Maltese cat was castrated when 3 months old. He was a pampered pet of a pharmacy, where he apparently received more than ample portions of food. Several weeks before death he was treated for an abscess that followed the bite of another cat, but he made an apparently uneventful recovery in the Raritan Hospital for Animals. He died when  $23\frac{1}{2}$  years old.

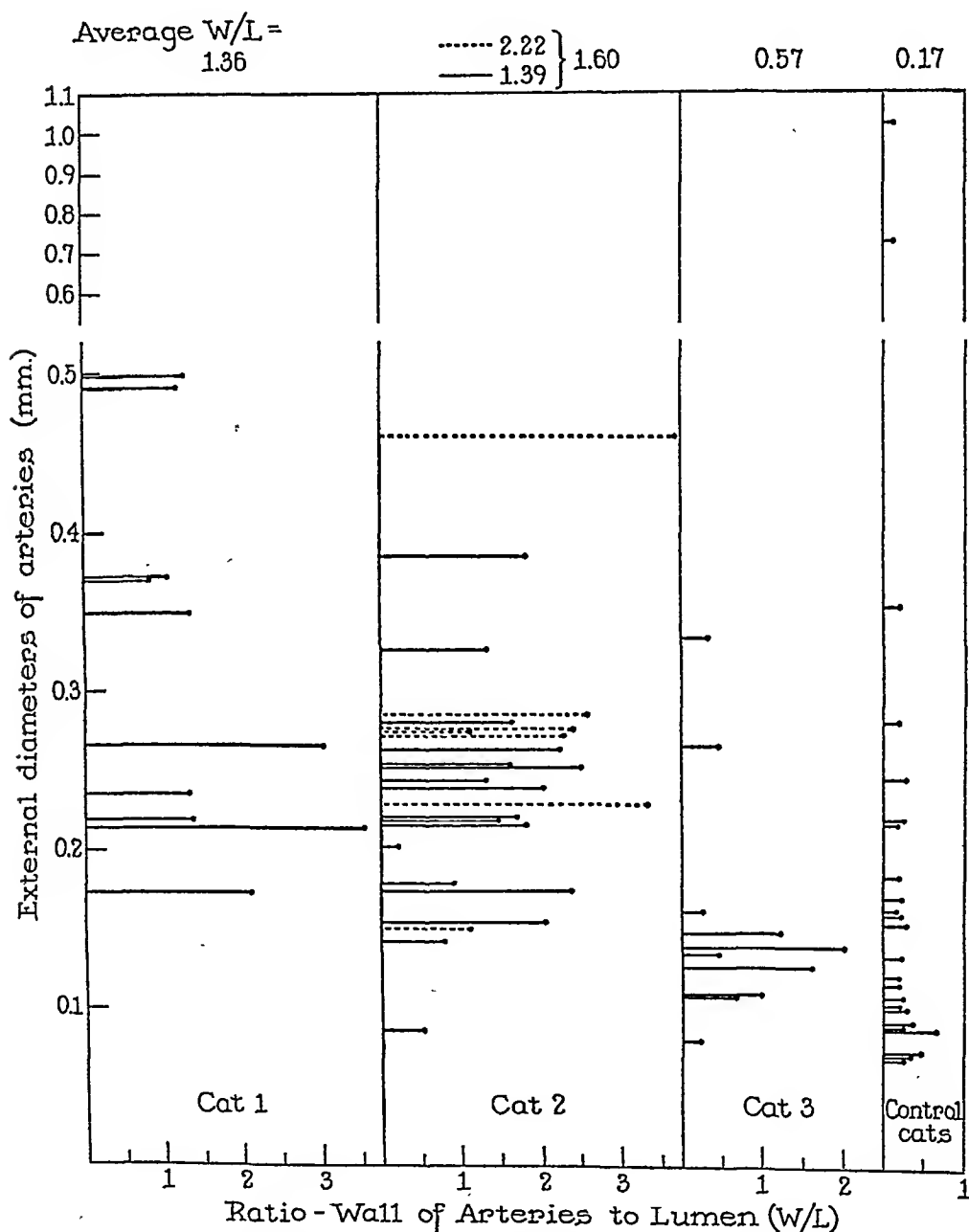
At autopsy (J. A. S.), the fur and teeth were in good condition. There were 50 cc. of green pus in the left pleural cavity, and the left lung was collapsed and covered with fibrinous exudate. There was some fibrin on the right pleural surface, but no evident pneumonia. On macroscopic section the bronchi of both lungs were prominent, and thickened arterial branches were recognized in all lobes.

On microscopic examination the left lung showed fibrinopurulent pleurisy, pulmonary abscess, acute bronchitis, and bronchopneumonia with early organization. The pleura overlying the right lung was covered by a little fibrin, but the parenchyma of this lung was almost completely expanded, and contained no exudate.

The arteries in the inflammatory and noninflammatory areas of the lungs showed a similar thickening of their walls. They were like those in cat 1, except that in this cat there was more connective tissue and smooth muscle inside the thick, circular layer of muscle. The average W:L ratio of the seven arteries in inflammatory tissue (indicated in Text-Fig. 1 by dotted lines) was 2.22. The W:L ratio of the 17 arteries in noninflammatory tissue (solid lines) was 1.39. It is postulated

that the slightly greater ratio in the arteries in the inflammatory areas may have been due to decrease in size of the lumina of the vessels by external pressure caused by the exudate, but the difference between the inflammatory and noninflammatory areas does not seem to be of great significance. The average W:L ratio of all 24 arteries was 1.60 (over nine times that in the control cats).

On gross and microscopic examination there were no changes in the thyroid, parathyroid, liver, pancreas, spleen, adrenal, kidneys, bladder,



Text-Fig. 1. The external diameter of each artery is plotted on the ordinates, the ratio of the wall to the lumen (W:L) on the abscissas. The vessels in inflammatory areas (cat 2) are indicated by dotted lines, those in noninflammatory areas by solid lines.



reproductive organs, pituitary body, brain, or bone marrow. There were lipochrome deposits at the nuclear poles of the myocardial cells, and slight scarring and thickening of the basement membrane of the glomeruli, but there were conspicuously few changes attributable to senility, as compared to what would be expected in men of corresponding age.

Thirty-five arteries from organs other than the lungs were measured and showed no variations from organ to organ. In only 3 arteries, each less than  $80\ \mu$  in external diameter, was the W:L ratio over 0.4, the greatest ratio being 0.585. The average W:L ratio of the 35 arteries was 0.164, almost exactly one-tenth of that in the pulmonary arteries of this cat.

### *Cat 3*

Cat 3 was a large male cat weighing 4.59 kg., with an unknown past history. It died following the same type of experiment as that for which cat 1 was used. Thickening of the pulmonary arteries was less advanced than in cats 1 and 2, but was significant. The measurements have been charted. The average W:L ratio was 0.57 (over three times the ratio found in controls).

### *Injection of Pulmonary Arteries in Cats 4 to 7, Inclusive*

We hoped by injection to study the distensibility of hyperplastic arteries. Unfortunately, the arteries of four cats injected were entirely normal, so that we obtained no knowledge of the behavior of hyperplastic vessels with this procedure. We injected the pulmonary arteries of two lobes of each cat with lead-gelatin at about  $40^{\circ}\text{C.}$  and then chilled the tissues, using the adaptation by Dock<sup>5</sup> of the method of injection devised for the coronary arteries by Schlesinger.<sup>6</sup> One of the two lobes injected in each instance had been previously distended by intratracheal pressure, while the pulmonary artery of the other was injected when the lobe was collapsed. Other tissue from the same pairs of lungs was studied without arterial injection, sometimes with, sometimes without previous pulmonary distention. The injected arteries, as would be expected, had very low W:L ratios, the average of the 24 measured being 0.026. The average W:L ratio of 14 uninjected arteries of these four cats was 0.17. The latter are charted with the other control cats (Text-Fig. 1).

### *Control Cats*

Slides were examined of tissue from the lungs of five additional cats (nos. 8, 9, 10, 11, and 12). In one (no. 11), no measurable arteries were found. Measurements of the other four are combined in Text-Figure 1, with those from the uninjected arteries of cats 4, 5, 6, and 7. The W:L ratio for 24 arteries from the eight cats was 0.17.

In a recent *human* autopsy (no. 11488) the pulmonary arteries and arterioles appeared to be much thicker than usual when examined without measurement. However, the W:L ratio of these vessels was only 0.13.

### DISCUSSION

It will be seen that the thickness of the pulmonary arteries as expressed by the W:L ratio had a different order of magnitude in cats 1 (1.36), 2 (1.60), and 3 (0.57) than in the remaining animals (0.17); also, that the W:L ratios of the extrapulmonary arteries in cat 2 (0.16) were much like those found in normal pulmonary arteries. In other words, in this animal at least, the changes were limited to the arteries in the lungs. The fact that all of the arteries in the sections studied were equally hyperplastic apparently shows that there was no beading of the arteries, as described by Ettinger<sup>2</sup> in the guinea-pig. Ettinger perfused the pulmonary artery of half-grown cats with Janus green, and found that there was enough constriction to reduce the perfusion rate by about 25 per cent. In a full-grown cat the constriction was enough to block the flow almost completely. The lung was sectioned immediately, and showed obliteration of the lumen of a pulmonary artery about 200  $\mu$  in external diameter. He interpreted the thickening of the arterial walls as due to the contraction of the vessel. Of the contraction there can be no doubt, but the enormous amount of smooth muscle shown in his illustrations must raise the question whether the constricted artery had not been extremely hyperplastic even before the perfusion. The picture in many ways resembles those found in our cats 1, 2, and 3, except that we did not find any separate bundles of longitudinal muscle in the adventitia of any of our animals. Wilens and Sproul<sup>7</sup> studied 487 rats kept over their entire natural life span. There were no constant morphologic changes with increasing age except in the coronary and pulmonary arteries. In the pulmonary arteries of almost every rat over 2 years of age there were degenerative changes, commonly with calcium deposition. They found "atrophy of the smooth muscle coat and replacement of fibrous tissue leading to irregular thickening of the wall. In less involved areas the smooth muscle often appeared hyperplastic." Smooth muscle hypertrophy in the media of a pulmonary artery is shown in their Figure 12. It resembles strongly that found in our cats 1, 2, and 3.

In the pulmonary arteries of our cats we found no lesions that we interpreted as atrophy. The smooth muscle cells were greatly enlarged and apparently increased in number. We interpret the picture as representing both hypertrophy and hyperplasia. There is no clear evidence in the animals studied that inflammation entered into the causation of the lesions. In the one case in which it was studied the heart was ap-

parently normal. On the other hand, although the muscle cells appeared large, there was no mitotic activity or other evidence of neoplasia. In other words, the cause of the process is entirely unknown.

The arterial change which we have described is apparently entirely unrelated to arteriosclerosis. Most reports on spontaneous arteriosclerosis in laboratory animals have dealt especially with changes in the aorta. However, Hueper<sup>8</sup> found cone-shaped calcified foci in the large and medium-sized branches of the pulmonary arteries in 12 of 75 rats examined. He considered these to be spontaneous. No lesions were found in the heart, aorta, liver, spleen, pancreas, adrenals, kidneys, or brain. In view of the experience of Hueper with degenerative lesions, and of Ettinger,<sup>1,2</sup> Wilens and Sproul,<sup>7</sup> and ourselves with the apparently very different hyperplastic changes, the possibility must be considered in the rat and cat that the preponderance of lesions in the pulmonary arteries may perhaps be related to the dependent position of the lungs in these animals. This possibility cannot be either established or denied with the data available.

### SUMMARY

In a very small percentage of cats, the pulmonary arteries show extreme hypertrophy and hyperplasia of the smooth muscle of the media. The cause of this lesion is unknown.

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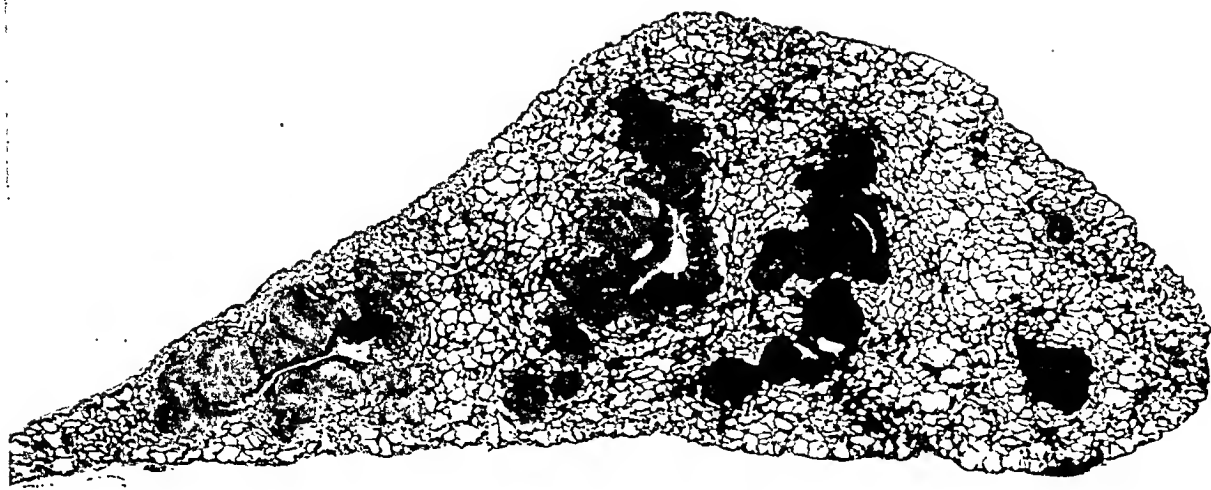
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### DESCRIPTION OF PLATE

#### PLATE 161

FIG. 1. Section of lung of cat 1. Nodular and confluent groups of arteries with marked hypertrophy of their walls. Hematoxylin and eosin stain.  $\times 11.5$ .

FIG. 2. The group of vessels below and to the right of the center of Figure 1 is shown at a higher magnification. The extreme hypertrophy of the arterial walls is limited to the media, and depends upon hypertrophy and hyperplasia of smooth muscle. Hematoxylin and eosin stain.  $\times 140$ .



1



2

Olcott, Saxton, and Modell

Medial Hyperplasia in Pulmonary Arteries



# CEPHALOTHORACOPAGUS MONOSYMMETROS

## REPORT OF A CASE \*

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Double human monsters occur so infrequently that statistics on their incidence are not particularly accurate. It is estimated that they occur once in about 50,000 births.<sup>1</sup> One of the more common forms of equal conjoined twins are the cephalothoracopagi, in which certain parts of the head, neck, thorax, and abdomen are shared. Following is a description of such a monstrosity.

## REPORT OF CASE

This monster was stillborn on March 6, 1945. Its mother was a white woman, 30 years old, who had had one previous pregnancy terminating in miscarriage at 11 weeks. The labor was difficult, lasting 25 hours. The vertex presentation was extracted manually. About 5 liters of amniotic fluid escaped during delivery. Birth occurred 4 weeks before the expected date of confinement. The mother was Rh positive.

### *Description*

The monster consisted of two female babies "joined" ventrally from the midportion of the abdomen upward (Figs. 1 and 2). It measured 36 cm. in length. It presented one head, one neck, one chest, and one upper abdomen formed by a fusion of two, two lower abdomens, two pelves, four arms, and four legs.

*Head.* The relatively large head had two faces. The faces were situated approximately at right angles to one another. It is convenient to speak of the aspect of the monster presenting the faces as the anterior aspect. There were apparently three eyes. The lateral eyes were normal, but the middle eye was common to the two faces and protruded about half way from its socket. This double eye was really two single eyes, except for a circular defect in the sclera where the two eyes were joined. There were two corneas which were 5 mm. apart. At the scleral defect the two retinas were in contact with one another.

Each face had a nose and a mouth and there were no malformations of the lips or palates. There were two ears, one located on the lateral aspect of each face.

The head was anencephalic. At the vertex there was a circular defect of the scalp and skull measuring about 5 cm. across. Through this a mass of friable, dark reddish tissue protruded. Similar tissue filled the small cranial cavity. Microscopically this consisted of highly vascular

\* This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the U. S. Navy.

Received for publication, August 9, 1945.

disorganized neuroglia. There was a prominent bony ridge extending along the floor of the calvarium from the sella turcica to the occipital protuberance. This divided the posterior cranial fossa into right and left halves. A foramen magnum in the bottom of each half led into right and left spinal canals. Two optic nerves arose in the double eye and soon became lost in the amorphous material filling the skull.

*Trunk and Extremities.* From each foramen magnum extended a vertebral column (Fig. 3). The neck was short and thick. The common chest was large, consisting of a part of the chest of each twin. The chests were joined by the ribs and no sterna were present. The posterior rib segments were shorter than the anterior ones; thus the anterior shoulders were 11 cm. apart and the posterior shoulders were only 3.5 cm. apart. Each anterior shoulder possessed a clavicle, but the posterior shoulders had none. The anterior chest showed two nipples; no nipples were seen posteriorly.

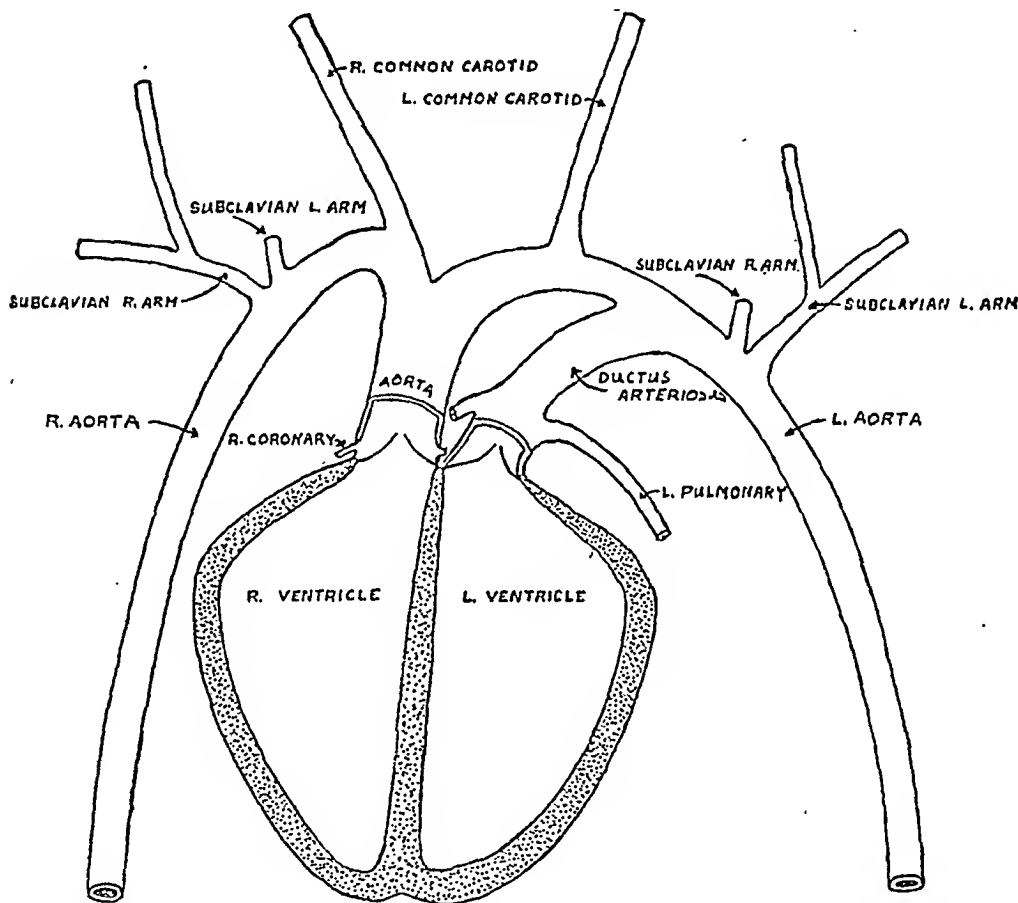
The abdomens were joined above the navel, but separate below. The navel and umbilical cord were single. The lower portion of each abdomen and the pelves appeared normal. Each twin had normally developed female external genitalia.

*Body Cavities.* The thorax contained one pericardial sac and two pleural cavities, one heart, and two lungs. The thorax and abdomen were separated by a single broad diaphragm. The abdominal organs consisted of one liver, one spleen, one stomach, one duodenum, two intestinal tracts, four adrenals, two complete urinary tracts, and two complete sets of female genitalia. The peritoneal cavities of the twins communicated above the umbilicus. There was a single lesser peritoneal sac behind the stomach.

*Cardiovascular System.* The heart was large and was located in the midline. The apex was deviated slightly to the right and showed a slight cleft where the right and left ventricles joined. The heart measured 4 cm. transversely, 4.5 cm. vertically, and 2 cm. anteroposteriorly. It had two auricles and two ventricles. The interventricular septum was intact. The interauricular septum was imperfect so that the auricles communicated with each other through a large foramen. The valves between the auricles and ventricles appeared normal.

The great vessels were transposed (Text-Fig. 1). The aorta arose from the right ventricle and the pulmonary trunk from the left ventricle. From the posterior part of the pulmonary trunk arose right and left pulmonary arteries; these vessels were small. The main part of the pulmonary trunk continued upward and to the left as the ductus arteriosus, and joined the left aorta between the left common carotid and subclavian arteries.

Right and left coronary arteries arose from the base of the aorta. The aortic trunk divided about 1 cm. above the heart into right and left aortas. From the left aorta arose the left common carotid artery and two subclavian arteries, one to each arm of the left twin. From the right aorta arose the right common carotid artery and two subclavian arteries, one to each arm of the right twin. Each descending aorta bore



Text-Fig. 1. Arterial system.

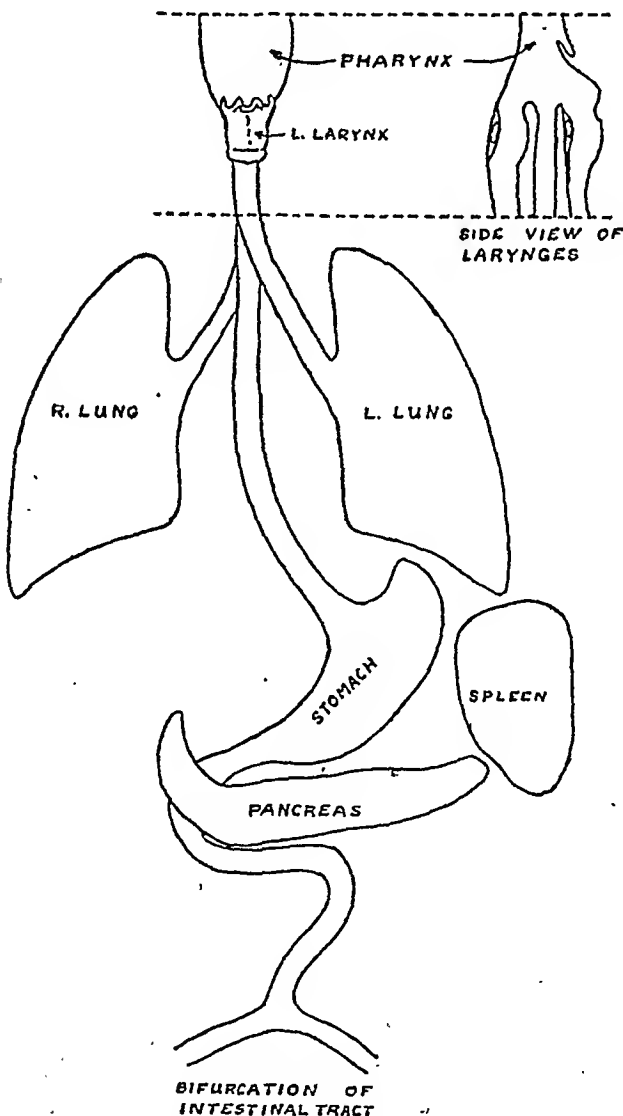
the usual relationship to the respective spinal column, and the lower arterial system for each twin was normal.

There were two inferior venae cavae which joined just below the liver. The large vena cava thus formed entered the heart at the junction of the two auricles posteriorly. Right and left pulmonary veins entered the left auricle. A single superior vena cava entered the upper part of the right auricle. This vessel was formed by the junction of right and left innominate veins. Each innominate vein received an internal jugular and two subclavian veins. A right azygos vein also emptied into the right auricle posteriorly.



The single umbilical cord contained one large vein and two pairs of arteries. The vein entered the anterior surface of the liver and continued into the common vena cava. A pair of umbilical arteries passed in the proper location to each twin.

*Respiratory System.* The structures concerned with respiration consisted of the two noses, a Y-shaped pharynx, two larynges, two tracheae, and two lungs. The upper limbs of the Y-shaped pharynx communicated with the noses. The limbs met in the lower part of the oral pharynx and the hypopharynx continued as a single esophagus and as two larynges. One larynx was located anterior to the esophagus and the other was posterior to it. Each larynx faced anteriorly, thus making the relations of the posterior larynx to the pharynx and esophagus rather peculiar (Text-Fig. 2). There was one hyoid bone, and only one



Text-Fig. 2. Respiratory and gastrointestinal systems.

thyroid gland was found. The trachea from the anterior larynx continued without bifurcation into the left lung, and that from the posterior larynx led to the right lung.

The left lung was smaller than the right. It had two principal lobes, each of which showed rudimentary fissures. The right lung was large and very irregular in shape. It had five lobes.

The posterior part of the mediastinum consisted simply of a thin septum formed by right and left parietal pleurae. The posterior margin of this septum joined the posterior thoracic wall about midway between the posterior rib junction and the left spinal column. The anterior part of the septum contained the esophagus and joined the pericardium about in the midline.

*Gastrointestinal System.* There were one esophagus, one stomach, and one duodenum. All of these were relatively normal in position when viewed from the front, but they did not bear their usual relationship to a spinal column. About 15 cm. distal to the duodenum the jejunum bifurcated, and beyond this there were two intestinal tracts, each consisting of a portion of small intestine, an appendix, and a colon. Each colon was located entirely in the lower abdomen and did not follow the usual course.

*Spleen.* There was a single spleen located in the upper part of the abdomen. It was rotated so that the diaphragmatic surface faced forward and inward. The artery which supplied it arose from the left aorta.

*Liver.* There was a single, large liver located in the midline in the upper abdominal cavity, but it extended to the umbilicus. Viewed anteriorly the liver was roughly triangular with apex downward and the right and left halves symmetrical. There were two transverse fissures across the lower half of the anterior surface and a vertical fissure from the apex to the upper transverse fissure. The umbilical vein entered through this vertical fissure just below the upper transverse fissure. The vertical fissure continued on the posterior surface, and from it the common bile duct left the liver about 2.5 cm. from the apex to enter the duodenum. No gallbladder was found. The liver measured 7.5 by 7 by 3 cm. Posteriorly there was a large caudate lobe measuring 3 by 3 by 1.5 cm. The inferior vena cava passed through the liver just anterior to the caudate lobe.

*Pancreas.* There was a single pancreas in the usual location. A small piece of pancreatic tissue extended anteriorly and to the right of the duodenum.

*Urinary Tracts.* Each twin had a complete urinary tract, so that there were four kidneys, four ureters, and two bladders. The kidneys were normal in size, shape, and position.

*Genitalia.* Each twin had complete female genitalia. The ovaries, tubes, uteri, and vaginae were normal.

*Nervous System.* No abnormalities were observed in the peripheral nerves. There were four sympathetic chains, one on each side of each vertebral column.

#### COMMENT AND COMPARISON

Obviously, conjoined twins are monozygotic. It is probable that monsters of this type occur when two primitive streaks forming on the ectodermal plate of a single developing ovum are in such close proximity in their cephalic and mid-portions that certain tissues subsequently developing from them are shared. In man, female double monsters are two or three times as frequent as males, although monozygotic male twins occur a little more frequently than females.<sup>1</sup>

Medical literature contains numerous descriptions of double monsters. A few similarities and differences between this case and others are recorded in the following paragraphs. A more extensive search would surely reveal cases even more closely resembling this one.

Cosmettatos<sup>2</sup> described a monster in which the head was anencephalic and presented two faces at right angles to one another. There were three eyes. The median eye possessed a single cornea and two optic nerves. Section revealed a scleral septum dividing the structure in two parts, each of which contained a retina and a lens. The body of this monster was essentially single although there were two vertebral columns as far as the sacrum.

A monster reported by Finola<sup>3</sup> had a single head with one face, but the external appearance of the double body closely resembled the case reported here. However, dissection revealed two complete sets of apparently normal thoracic organs. There was an arterial communication between the arches of the two aortas. The abdominal portion contained two sets of viscera, except for the gastrointestinal tract, which was single to a point 10 cm. proximal to the ileocecal valve where bifurcation occurred. A pancreas flanked each side of the duodenum, and the biliary tracts from the two livers emptied into opposite sides of the duodenum. Scammon<sup>1</sup> suggested that in monsters of this type the digestive tube is single to the level of the origin of the yolk stalk from the small intestine, and double beyond.

In a case of two conjoined fetuses, occurring in a set of monozygotic triplets reported by Messinger and Shryock,<sup>4</sup> the union occurred at the thorax and upper abdomen. The heart was a single four-chambered structure, and the umbilical cord contained two arteries and two veins. The livers were fused and cystic. The upper portion of the gut was

single and the lower portion was double. There was incomplete rotation and fixation of the colon with transposition of the colon in one fetus.

According to Jordan and Kindred,<sup>5</sup> the viscera in conjoined twins are frequently arranged in mirror image fashion. When this occurs it is always the right hand twin that shows the situs inversus viscerum. In the present case the fixation of the colons was so faulty that it was not possible to determine whether one was transposed.

In a double ovine monster described by Goss and Cole<sup>6</sup> the heart and great vessels were quite similar to those structures in the monster reported here. However, the great vessels were not transposed, and the right pulmonary artery arose from the right aorta instead of from the pulmonary trunk.

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[ Illustrations follow ]

## DESCRIPTION OF PLATES

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### PLATE 162

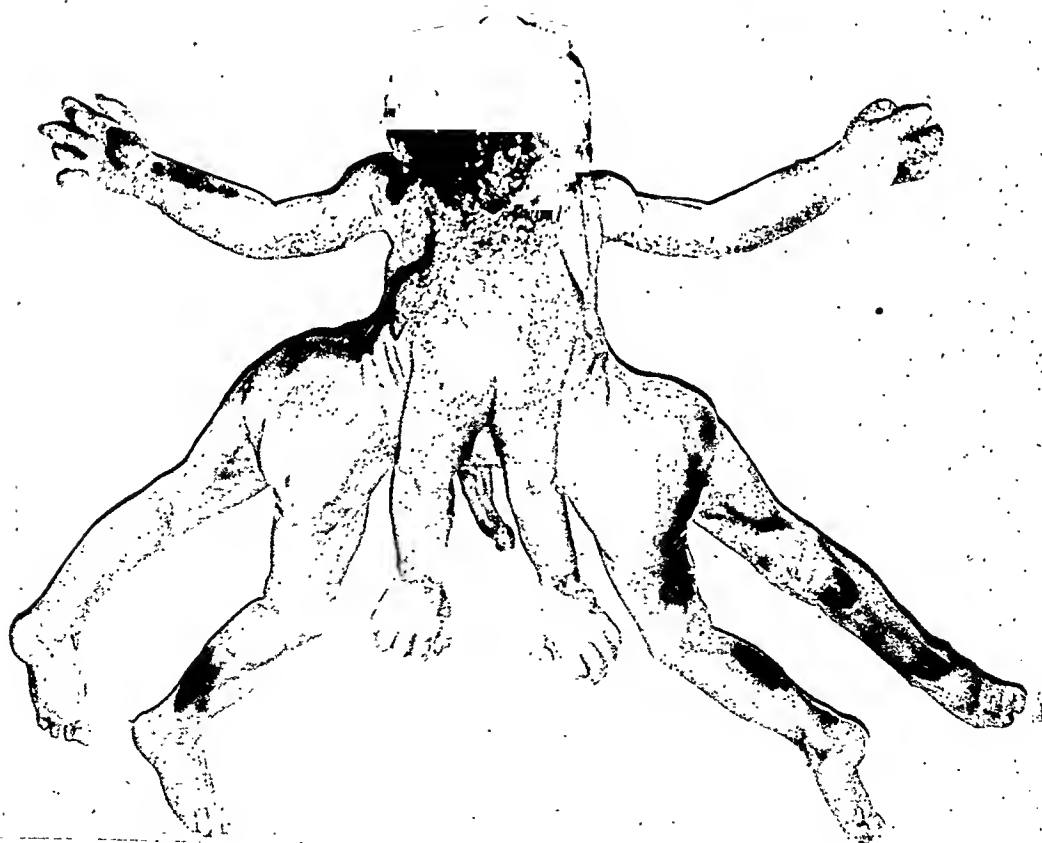
FIG. 1. Anterior view of cephalothoracopagus monosymmetros.

FIG. 2. Posterior view of cephalothoracopagus monosymmetros.

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Gunter

Cephalothoracopagus Monosymmetros

PLATE 163

FIG. 3. Roentgenogram of the cephalothoracopagus monosymmetros illustrated in the preceding figures.

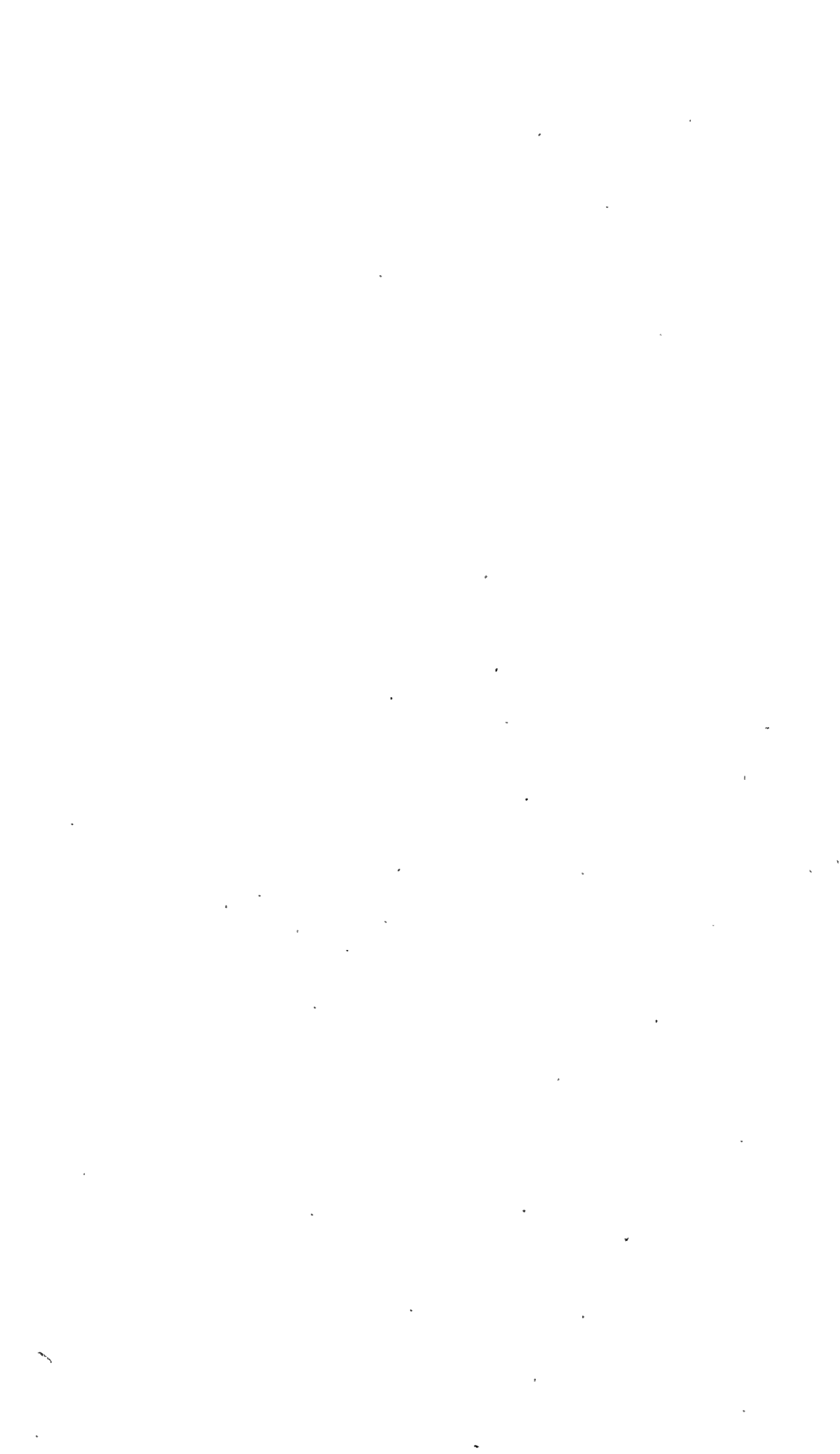


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Cephalothoracopagus Monosymmetros

Gunter





# THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XXII

SEPTEMBER, 1946

NUMBER 5

## THE FULMINANT FORM OF EPIDEMIC HEPATITIS \*

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\* Received for publication, May 8, 1946.

## INTRODUCTION

Epidemic hepatitis has attained pandemic proportions during this war. Large outbreaks have occurred in many parts of the world and in the armies of a number of nations.<sup>1-23</sup> The pathology of this disease as observed in the Army of the United States during the epidemic of 1942 has been dealt with in previous papers.<sup>24, 25</sup> More recently, an acute form of epidemic hepatitis of intense severity terminating fatally in less than 10 days has become prevalent; this we have termed the fulminant form. In a new series of 196 fatal cases occurring between August, 1943, and April, 1945, which we have studied at the Army Institute of Pathology, over half fall into this category. By contrast, in the previous series not a single equally fulminant case was encountered, and only one was reported in the great Swedish epidemic of 1927;<sup>26</sup> the usual duration of the fatal disease was then from 4 to 6 weeks; in other words, the course of fatal hepatitis was predominantly subacute. These divergences in duration reflect striking differences in the pertinent pathologic changes. Thus, in the more fulminant form, the parenchyma of the liver is destroyed completely and uniformly, and this destructive process is accompanied by an intense inflammatory reaction. In the more subacute form seen in 1942 and also in approximately one-fourth of the 1943-1945 series, destruction of the liver is incomplete, the involvement characteristically not uniform, regenerative hyperplasia of surviving parenchyma leads to the production of much new tissue, and inflammation is less pronounced.

Another significant difference is in the epidemiology. In 1942, hepatitis in many instances followed administration of yellow fever vaccine containing human serum. In the new series, such vaccine had not been used; but nearly one-half of the patients had sustained combat trauma. Since seriously wounded patients customarily received transfusion of whole blood, serum, or plasma, it may be assumed that a high proportion of the wounded in this series were thus treated. But it is not known in how many the causal agent of hepatitis was introduced by therapeutic procedures, especially as in several theaters of war large epidemics of hepatitis were prevalent. It is, therefore, an assumption to regard all the wounded cases as examples of "homologous serum hepatitis." This assumption is justified largely by the relatively long interval between date of wound (and presumably of first transfusion) and the clinical manifestation of the disease. The non-wounded cases, comprising approximately one-half of the series, represent both the epidemic and the endemic variants of "naturally" occurring hepatitis.

This study complements the previous report on the pathology of epi-

demic hepatitis<sup>24</sup> and gives a more comprehensive picture of the disease. It is based primarily on 94 fulminant cases in which the clinical duration did not exceed 9 days; however, 39 others with duration of from 10 to 19 days have been used to supply additional information on certain aspects of the disease. This latter group contains many examples indistinguishable from the fulminant form.

The scope and arrangement of this paper are outlined in the table of contents. The methods of study were similar to those employed in the earlier investigations at the Army Institute of Pathology.

### *Duration of Fatal Hepatitis*

Of the 196 cases studied, the duration of the disease could be determined with fair accuracy in 178. In the remainder the records were inadequate, or the discrepancy between the clinical accounts and the

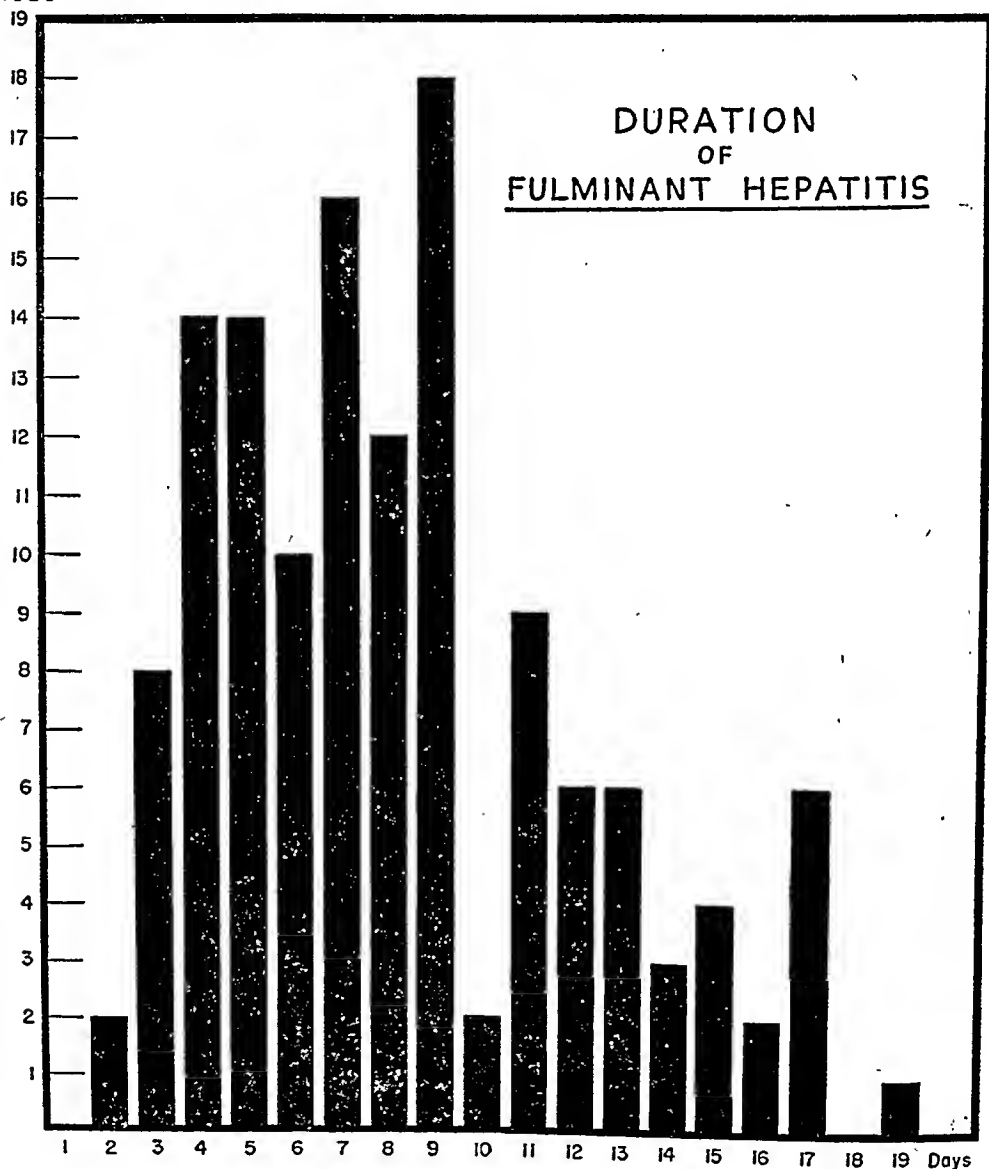
TABLE I  
*Duration of Fatal Hepatitis*

Old series: 118 cases (1942 to July, 1943)			New series: 178 cases (August, 1943, to April, 1945)	
Duration (Days)	No. of cases	Per cent of cases	No. of cases	Per cent of cases
Less than 10	0	0	94	53
10-19	14	11	39	21
20-29	20	16	10	6
30-39	31	26	13	7
40-49	20	17	4	2
50-59	8	7	2	1
60-69	8	7	1	0.5
70-79	9	8	3	2
80-89	0	0	1	0.5
90-100	5	5	3	2
Over 100	3	3	8	4

character of the lesions was so great that this group was excluded from analysis. The pertinent data for the series are given in Table I. It will be noted that in 53 per cent the course of the disease, from beginning of symptoms to death, was less than 10 days, and that in 74 per cent the disease terminated fatally within 20 days. A more detailed analysis of this group is shown graphically on a day-by-day basis in Text-Figure 1, where the vertical columns represent the number of cases and the abscissae the corresponding duration of disease. Inspection of the graph brings out, first, that the number of cases in which death occurred within so short a time as 4 days is considerable (21 per cent); second, that the number of fatalities on any one day between the fourth and the ninth is greater than the number for any subsequent day; and third, that there is a sharp drop in the number of deaths beyond the

ninth day which marks a turning point. This impression is strengthened by the character of the lesions: in almost all of the cases which ended fatally before the tenth day, destruction of liver cells was complete or nearly so, whereas in those with a longer survival period, de-

NO. OF  
CASES



Text-Fig. 1. Duration of the disease in 133 cases of fulminant hepatitis.

struction usually was incomplete. It is for this reason that we have considered the cases terminating in less than 10 days as representing a fairly homogeneous type, namely, the fulminant form of hepatitis.

Comparison of the duration in the present series with that of the epidemics of 1942 in the United States Army, and of 1927 in the civilian population of Sweden brings out marked differences which have al-

ready been indicated. Suffice it to state that the median duration in the present series is 8 days, whereas in the other epidemics it exceeded 5 weeks.

The search for the elements responsible for the more fulminant course of hepatitis during the past 2 years is a cardinal task of this investigation.

### *Incidence of Previous Trauma and Transfusion*

Factors that can clearly be correlated with duration of disease are previous trauma and subsequent transfusions of blood (Table II). In nearly all cases trauma—combat wounds or burns—was of serious

TABLE II  
*Incidence of Previous Trauma (Wound or Burn) in 178 Cases of Fatal Hepatitis (New Series)*

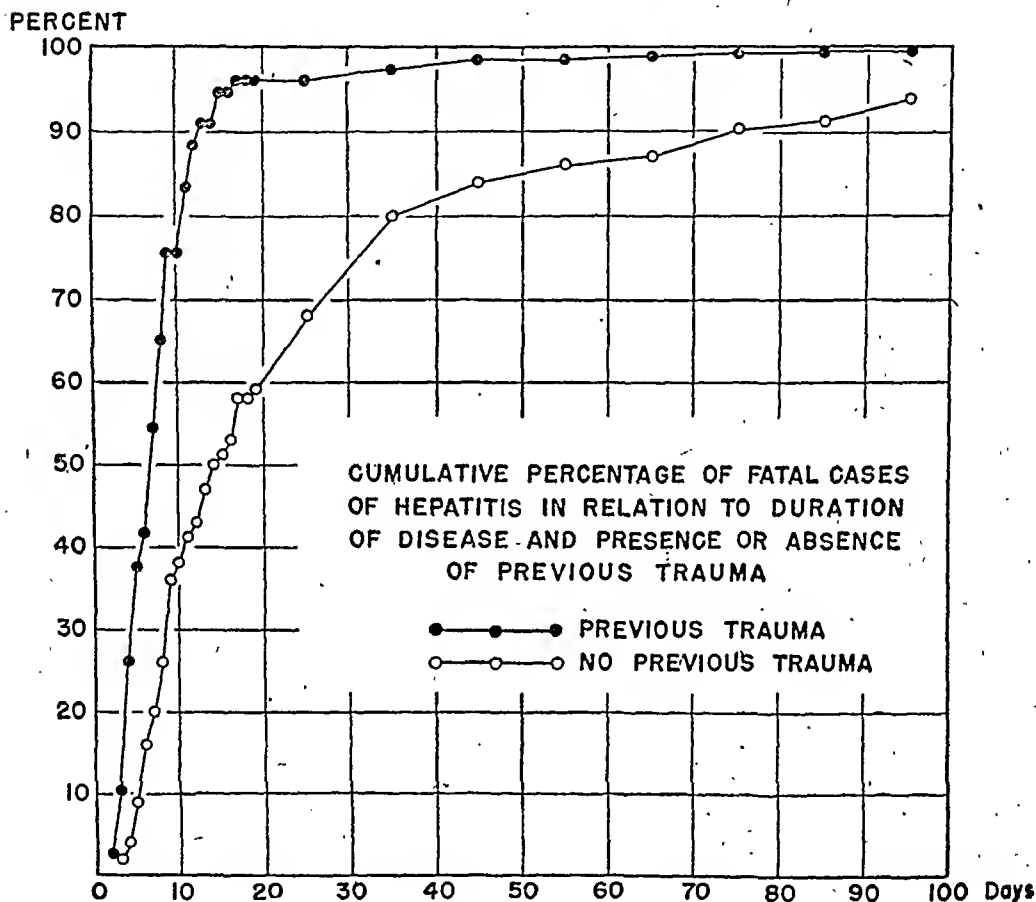
Duration of disease (Days)	Wounded	Burned	No previous trauma	Total number of cases
Less than 10	52 (55%)	6 (6%)	36 (38%)	94
10—19	16 (41%)	0	23 (59%)	39
20 and over	3 (7%)	0	42 (93%)	45
Total no. of cases	71 (40%)	6 (3%)	101 (57%)	178

degree and was sustained within 4 months of the onset of hepatitis. Most of the wounded received transfusions of blood or its derivatives within a few days of injury: hence these cases probably may be regarded as examples of homologous serum hepatitis. It is from this premise that we here analyze the relation of trauma to the duration of fatal hepatitis.

Consideration of Table II reveals the significant fact that the patients with hepatitis who had previously sustained trauma succumbed much sooner than those who had not been injured. Thus, while there was history of trauma in 61 per cent of the total group in which death occurred within 10 days, the incidence dropped to 41 per cent for the group with a duration of from 10 to 19 days, and to only 7 per cent when survival exceeded 20 days. Conversely, column 4 of the table shows a progressive increase in nontraumatic cases in proportion to duration. These variations are graphically represented in Text-Figure 2, where the cumulative percentage of fatal cases has been plotted in relation to duration of disease and presence or absence of previous trauma. Here it is shown that 76 per cent of those cases with a history of trauma ended fatally within 10 days of the onset of symptoms, and

96 per cent by the 20th day, whereas the corresponding percentages for the nontraumatic group are 38 and 59.

Computation of the median duration of hepatitis in the two groups gives 7 days for the wounded and 15 days, or twice as long, for the group without trauma. The contrast between the two groups is shown graphically on a day-by-day basis in Text-Figure 3 using the same



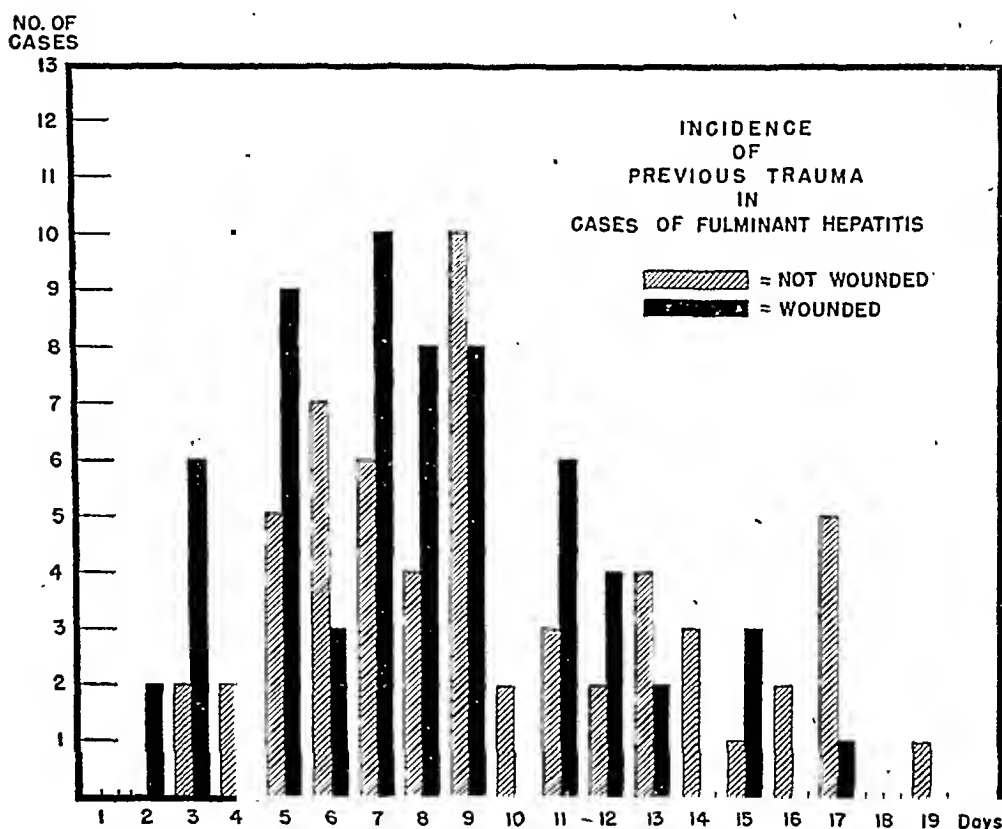
Text-Figure 2

data from which Text-Figure 2 was constructed. The graphs show that the course in the wounded group tends to be more fulminant than in the nonwounded; the mortality of patients with a history of trauma and transfusion was five times as great during the first 4 days after onset of symptoms. Evidence favors the conclusion that the type of fatal hepatitis which may follow trauma and transfusions of blood, *i.e.*, homologous serum hepatitis, tends to run a considerably more rapid course than does the naturally occurring disease.

#### *Distribution of Cases According to Age and Race*

The data from 130 cases of hepatitis in which the clinical course was less than 20 days are given in Table III. All were male soldiers (in-

cluding 7 Italian and German prisoners of war); 125 were white, 3 Negroes, one a Javanese, and one an American-born Japanese. The distribution is similar to that in the 1942 epidemic, and to that in a recent survey of 1,762 patients under treatment for hepatitis in Army General Hospitals.<sup>27</sup> It is almost exactly in direct ratio to the population in the Army. Factors of age, sex, or race, therefore, do not ac-



Text-Fig. 3. Comparative mortality on a day-by-day basis between nonwounded and wounded patients with fulminant hepatitis.

count for the preponderance of a more fulminant form of hepatitis in the present series.

TABLE III  
*Age in 130 Cases of Hepatitis in Which the Duration of the Disease Did Not Exceed 19 Days*

Years	No. of cases	Per cent of cases
Less than 20	10	8
20-24	45	35
25-29	41	32
30-34	25	18
35-39	6	5
Over 40	3	2



*Geographic Distribution and Epidemiologic Types of Fatal Hepatitis*

In order to determine possible variations of the disease in relation to climate and other factors, the cases in the present series have been tabulated according to geographic location and duration of disease, and subdivided as wounded and nonwounded groups in each theater of war (Table IV). In this tabulation the European theater includes Great Britain and the continent except the Italian peninsula; the Mediterranean theater includes Italy, Africa, and the Middle East; the Pacific theaters include the islands of the Pacific, and India, China, and Burma. It will be noted that 90 per cent of the cases came from combat areas and only 10 per cent from the United States.

TABLE IV  
*Geographic Distribution of 178 Fatal Cases of Hepatitis  
(New Series)*

Duration of disease	United States		European theater		Mediterranean theater		Pacific theaters	
	Wounded	Not wounded	Wounded	Not wounded	Wounded	Not wounded	Wounded	Not wounded
(Days)								
Less than 10	2	5	31	2	20	21	5	8
10—19	0	4	11	5	2	6	3	8
20 and over	0	7	1	6	2	8	0	21
Total	2	16	43	13	24	35	8	37

During the period covered by this study, extensive epidemics of hepatitis existed in the Mediterranean and Pacific areas, and were absent (except during the final months) in the European theater and in the United States. Cases in the first two areas may, therefore, be regarded as belonging predominantly to the epidemic type; in the latter areas, to the endemic or sporadic type.

In the wounded group each casualty has been credited to the theater in which the injury occurred, although the first symptoms of hepatitis often did not develop until after evacuation to the United States.

The cases in the series may, with certain assumptions, be classified on the basis of epidemiology. There were 29 examples of the endemic and 72 of the epidemic type of the "naturally" occurring disease, and 77 of homologous serum hepatitis, most of the latter coming from the European theater. Cases without trauma predominated slightly in those from the Mediterranean theater and strikingly so in those from the Pacific.

*Mortality*

In all recorded epidemics of hepatitis the mortality has been low, ranging from 0.2 to 0.4 per cent. Thus, during the United States Army epidemic of 1942 there were 51,337 cases reported,<sup>11</sup> with a mortality rate of 0.24 per cent. During the more recent Army epidemics, 68,000

cases (in round numbers) and 196 deaths have been recorded; that is to say, the death rate was 0.3 per cent. In view of the change in character of fatal hepatitis during recent years the agreement in mortality rates is surprising.

It should be emphasized, however, that the rate for the 1943-45 epidemics is based upon incomplete returns and must be regarded as an approximation. Actually, the mortality of "naturally" occurring hepatitis is probably lower, whereas that of homologous serum hepatitis may be higher. No precise information is as yet obtainable on either variant. Estimates are based largely upon cases which have been hospitalized; no account usually is taken of the mild and frequently nonicteric forms of the disease which escape recognition. For homologous serum hepatitis, attempts to determine case mortality have been particularly unsatisfactory, but the impression is that it may be several times as high as in the naturally occurring disease. The mortality of 0.3 per cent in the epidemics under discussion is an average, and has varied in different localities and at different times.

With regard to duration of the endemic type, it is interesting to note that there were 16 acute and 13 subacute fatal cases (defining the latter as running a course of more than 20 days); they were almost equally divided between the United States and Europe. In the epidemic group, subacute cases slightly outnumbered acute cases in the Pacific theater (21 to 16), whereas in the Mediterranean area the proportions were more than reversed (8 to 27).

No correlation was apparent between duration of illness and geographic location in the endemic type. For the epidemic type, however, the disease was predominantly acute in the Mediterranean and subacute in the Pacific areas. Measured by duration it appears to have been more virulent in the Mediterranean than in the Pacific areas.

In the homologous serum group, practically all cases ran an acute course without any apparent influence by geographic factors; only 3 were subacute. It would be a mistake, however, to assume at once that this is a general characteristic of this type of hepatitis.

#### CLINICAL PICTURE OF FULMINANT HEPATITIS

A number of recent reports by American,<sup>11-18</sup> British,<sup>4-10</sup> and German<sup>19-23</sup> observers have dealt with the clinical picture of epidemic hepatitis in its usual benign form ending in recovery. Wherever the disease has occurred, the clinical pattern has been remarkably uniform; the symptoms and signs have varied in degree, not in kind.<sup>28</sup> There is an impression, however, that in recent years hepatitis has become more severe.

In this paper we are concerned with a fatal form in which nearly all

liver cells are destroyed within a few days. In an effort to correlate lesions and functional disturbances an analysis has been made of the clinical manifestations of the fulminant cases. The analysis is based primarily on the 94 cases of that group, but other cases are incorporated in certain sections since their clinical and anatomic manifestations were identical even though survival was longer (10 to 19 days). The general data have been augmented by abstracts of the clinical records of representative cases.

### *Similarity between "Spontaneous" and "Inoculation" Hepatitis*

A question of great importance arose in connection with the analysis: Is "naturally" occurring fulminant hepatitis, as seen in either its endemic or epidemic variants, clinically distinguishable from the disease "artificially" induced by parenteral injection of blood or its derivatives? The answer can readily be given: Except for a tendency to a more rapid course in serum hepatitis, no differences were discernible. This finding is in accord with observations on nonfatal cases.<sup>3,9,19,20,29-33</sup> It is, therefore, permissible to combine the two groups for purposes of analysis.

### *Course of Fulminant Hepatitis*

The three phases previously reported in the subacute form can usually be recognized in the fulminant disease: (1) an initial, prodromal or pre-icteric stage; (2) an intermediate stage which begins with the onset of jaundice, and (3) a final stage which generally is ushered in abruptly by grave nervous manifestations. The time relations were less sharply defined in the fulminant than in more protracted cases. The pre-icteric period varied greatly (Text-Fig. 4) but was generally 2 or 3 days. The duration of the terminal phase did not differ significantly from that of the previous series, ranging from 1 to 4 days. Since the total duration in the fulminant group was less than 10 days, it is evident that the intermediate stage was often very brief; indeed, in some instances the manifestations of the prodromal and final stages merged.

*Initial Symptoms.* Two usual combinations of initial symptoms may be recognized: (1) An "infectious" type in which the disease is ushered in suddenly by the symptoms common to many acute infections: fever, chilliness, or, more infrequently, shaking chills, malaise, generalized aching, pains in the joints, back, and eyes, with gastrointestinal symptoms usually following in a day or so but rarely dominating the picture. (2) A "gastrointestinal" type in which the initial symptoms are similar to those encountered in the subacute variety of the disease: anorexia, nausea, epigastric discomfort or pain, with variable elevation in tem-

perature. The onset usually is more gradual with the second than with the first group; but it may be acute with severe nausea and vomiting from the start.

Sometimes initial symptoms of both types appear simultaneously, with neither in the foreground. Rarely, the disease begins with mental disturbances. The distribution of these several types of onset is shown in Table V which is based upon the fulminant cases and 19 others of more than 9 days' duration but with similar manifestations. It will be seen that four-fifths of the cases belong to the two main groups, which, roughly, are represented equally.

The 3 cases in which cerebral symptoms ushered in the disease deserve special mention. An indication of the extent to which central

TABLE V

*Predominant Types of Initial Symptoms in 113 Cases of Hepatitis in Which the Duration Did Not Exceed 19 Days*

Symptoms	No. of cases	Per cent of cases
Gastrointestinal type	48	43
Acute infectious type	40	36
Mixed gastrointestinal and acute infectious type	22	19
Mental disturbances	3	3

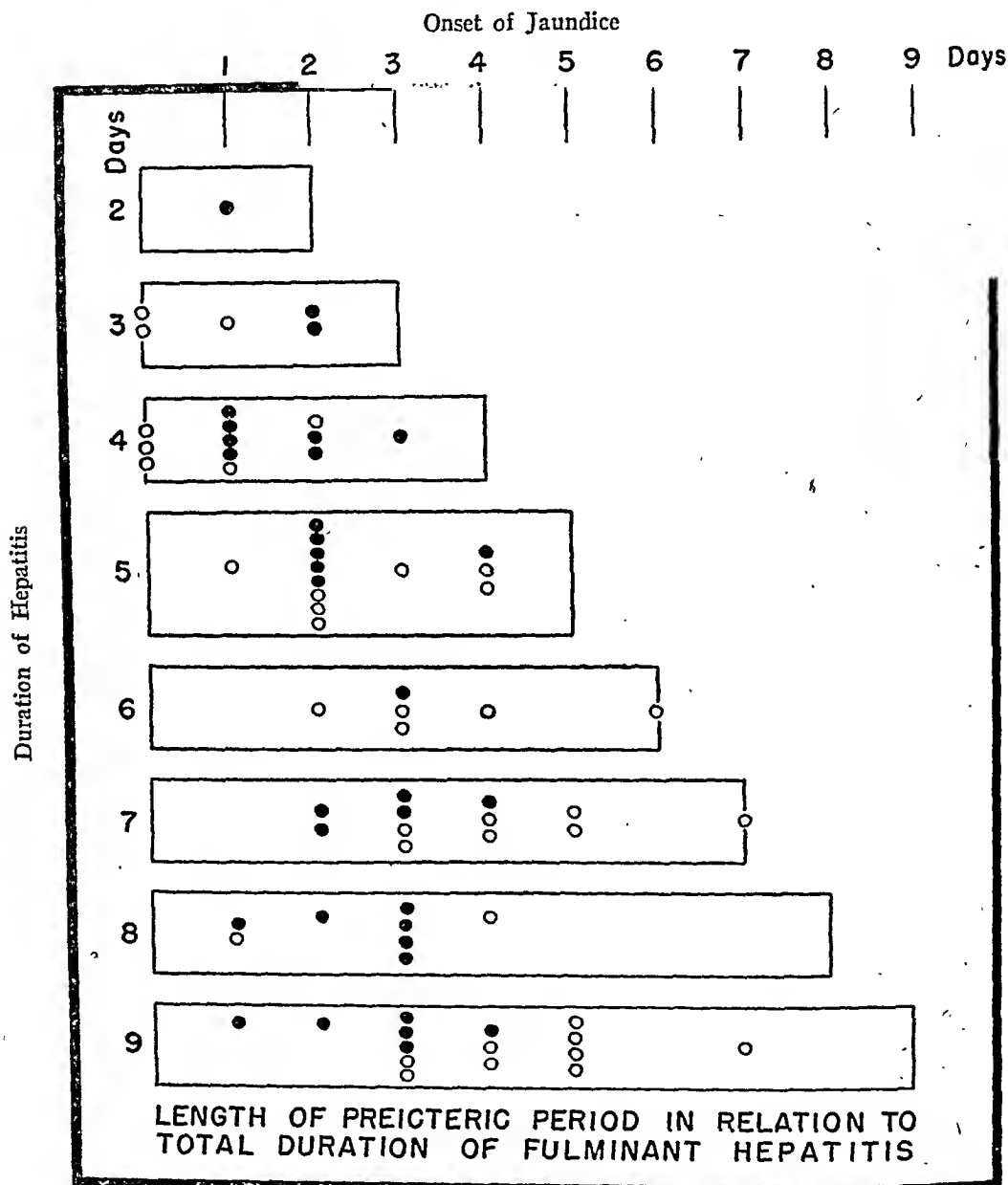
nervous manifestations may dominate the clinical picture is afforded by the fact that exploratory craniotomy was performed in 2 of these cases.

The prodromal symptoms observed in fulminant cases do not differ significantly in type or severity from those of the usual nonfatal cases. The predominant initial symptoms of the fulminant cases, as of those with recovery, have been independent of locality, and those of the two main types have coexisted during the same periods of epidemics although their relative frequency has varied. Thus, in an American Army Hospital in Italy, the "infectious" type occurred in 61 per cent, the "gastrointestinal" form in 39 per cent; <sup>18</sup> in a British garrison at Malta the proportions were reversed, only 37 per cent exhibiting the febrile type; <sup>6</sup> during an epidemic in the Middle East the two were represented equally.<sup>34</sup> The symptoms and signs of the febrile type have been described as closely resembling malaria,<sup>10,36</sup> influenza,<sup>2,35</sup> sand fly fever,<sup>6,8</sup> and "acute surgical abdomen."<sup>4</sup>

The observation that the subsequent clinical manifestations bear no relation to the character of initial symptoms is true for the fatal as well as for the nonfatal form of hepatitis.<sup>34</sup>

*Onset of Jaundice.* In Text-Figure 4 is shown the relation between

the onset of jaundice and the duration of disease in 72 fulminant cases. Those previously wounded (34 in number) are represented by solid circles; the nonwounded (38 in number), by open circles. The graph brings out several points of interest. First, it shows no significant dif-



Rectangles = duration of hepatitis; solid circles = onset of jaundice in wounded cases; open circles = onset of jaundice in nonwounded cases.

Text-Figure 4

ference in time of appearance of jaundice in wounded and nonwounded cases; in both the "spontaneous" and the "inoculation" variants there is considerable scattering. In several instances jaundice occurred at the onset of symptoms; in others it was delayed until the day of death; but on an average it appeared on the second or third day after the be-

ginning of symptoms. A positive correlation between onset of jaundice and duration of disease seems to be indicated by early jaundice in the more fulminant cases.

The time of appearance of jaundice and its subsequent intensity bore no relation to the type of initial symptoms. This same lack of correlation has been reported in nonfatal cases.<sup>18,36</sup>

*Final Stage.* The main features of the terminal phase were not appreciably different from those described in the earlier series. Usually a rather abrupt change for the worse was initiated by cerebral symptoms beginning with listlessness, drowsiness, increasing apathy; with restlessness, incoherence, disorientation; or with irritability and failure to cooperate. The symptoms of excitement often progressed to an acute maniacal state necessitating restraint. Frequently apathy and excitement alternated, but in the majority of cases deep coma eventually supervened. With the onset of cerebral symptoms hyperactivity of certain reflexes was the rule; a positive Babinski sign and persistent ankle clonus were common. Muscular twitching was frequent, while opisthotonos and generalized convulsions were occasional.

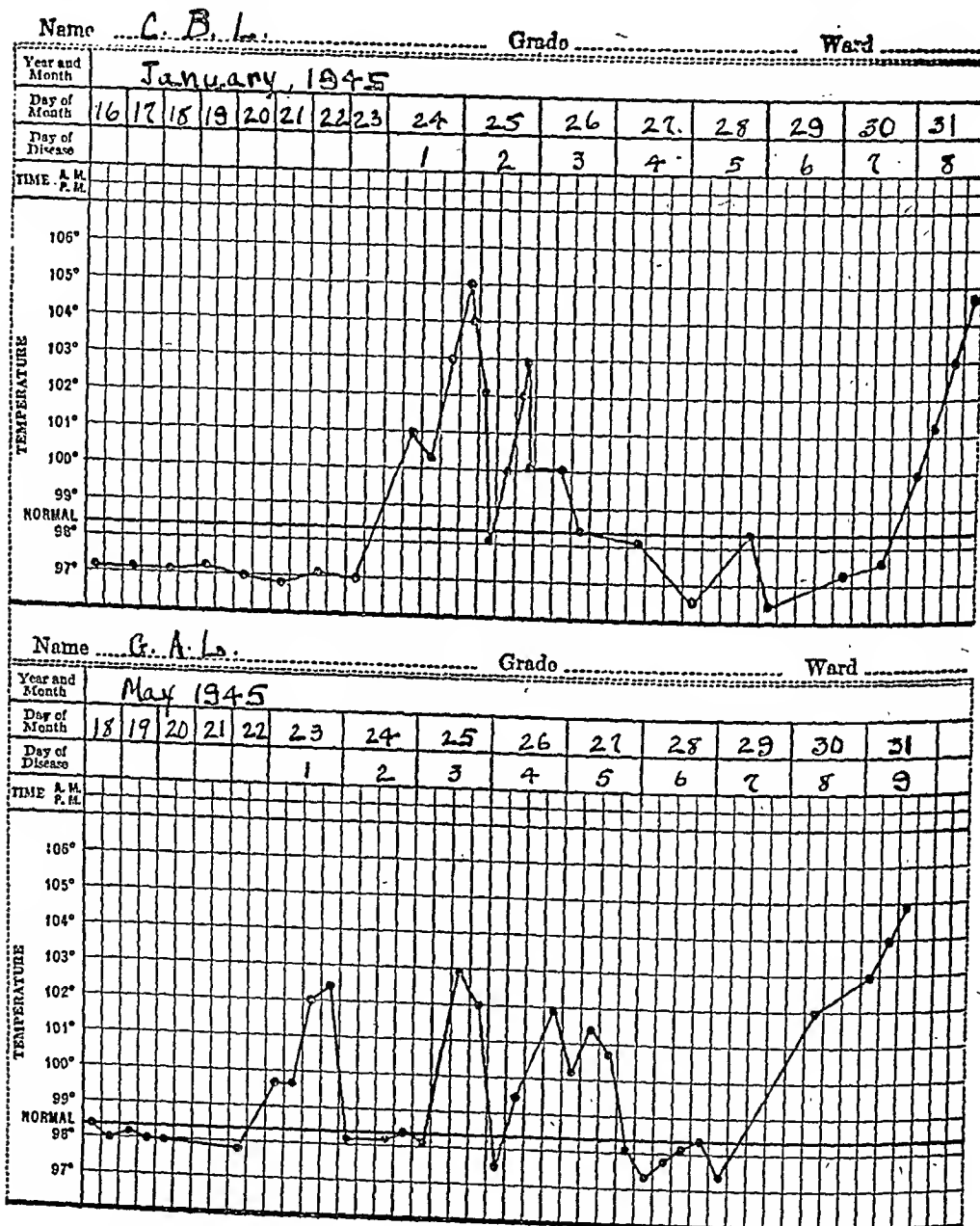
During the final phase jaundice often deepened rapidly. Vomiting frequently was severe and sometimes projectile. Bleeding from the gastrointestinal mucosa was evidenced by coffee-ground vomitus and melena, and was occasionally associated with purpuric phenomena or with frank gastrointestinal hemorrhage of considerable magnitude. Signs of pulmonary edema or patches of consolidation were found in the majority. Shock developed in a number of cases; in all there was a terminal rise of temperature.

### *Symptoms, Signs, and Laboratory Findings*

The analysis of the data included the following features: temperature, pulse rate, palpation of liver and spleen, recurrence of disease, jaundice, icterus index, total and differential leukocyte count, plasma proteins, urine, nonprotein nitrogen and urea nitrogen of blood, and blood sugar. Abstracts of representative clinical records illustrate these data.

*Temperature.* Temperature records for the prodromal stage were available in 68 fulminant cases; in all but one fever ranged from 99° to 104° F. and averaged 102° F. The peak usually was attained on the second day. With the onset of jaundice the temperature, as a rule, fell to normal or slightly below normal (96° to 97° F.) and remained so for several days, but in about one-fourth of the cases irregular subsequent rises took place. During the final phase the temperature almost invariably rose sharply, usually to 103° to 105° F., occasionally

to as high as  $107^{\circ}$ . Representative temperature curves are given in Text-Figure 5. Except for the terminal fever, the temperature reactions in fulminant hepatitis are similar to those in nonfatal cases;

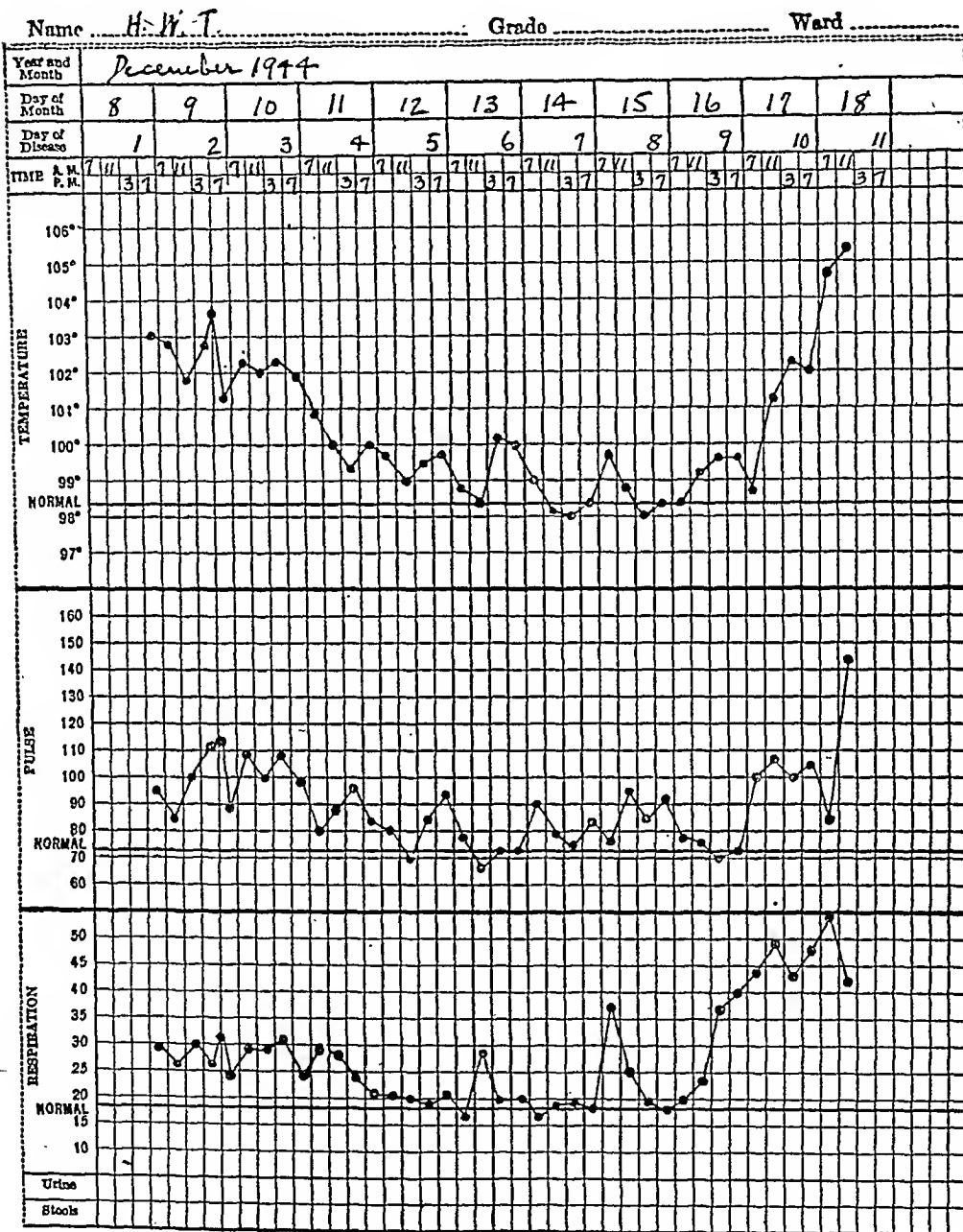


Text-Fig. 5. Representative temperature curves in cases of fulminant hepatitis.

most observers have reported a fever which was often high at the onset <sup>2,4,6,8,9,16,18,19,34,36,37</sup> and usually declined as jaundice appeared, although sometimes it was protracted. <sup>3,10,34,37</sup>

**Pulse Rate.** The pulse rate was nearly always elevated during febrile periods (Text-Fig. 6). Bradycardia was observed only in rare instances.

*Palpation of Liver and Spleen.* The liver was moderately enlarged and usually tender during the late prodromal and early icteric stages; as the disease progressed shrinkage was frequent but not invariable.



Text-Fig. 6. Clinical chart of a case of fulminant hepatitis with death on the eleventh day. Pulse rate rose during periods of fever.

Enlargement of the spleen was less constant; it was recorded throughout all stages in approximately 25 per cent of the cases. More precise statements concerning the sizes of these organs have little meaning because of the variability at different stages of the disease. In nonfatal



cases enlargement of the liver has been reported invariably when the disease was present in moderate or severe form, and frequently when it was mild; <sup>37</sup> other writers have recorded enlargement in the majority.<sup>8,13,16,18,19,34</sup>

For the spleen the figures usually given are 10 per cent or less; <sup>13,14,16,18,34</sup> although some observers have found readily palpable spleens more frequently, *i.e.*, in 13.6 per cent,<sup>37</sup> in 27 per cent,<sup>8</sup> or "usually."<sup>22</sup>

*Recurrence.* It is generally thought that a high degree of immunity is conferred by an attack of epidemic hepatitis, although accurate information is not available.<sup>33</sup> When second attacks do occur they are perhaps induced by nonspecific factors, by immunologically different strains, or by large doses of the infectious agent.<sup>5</sup>

TABLE VI  
*Icterus Index in 60 Cases of Fulminant Hepatitis*

Icterus index	No. of cases	Per cent of cases
Below 50	9	15
50 — 99	31	53
100—149	15	24
150—over	5	8

In the present series 4 patients had a history of a previous attack within 3 years of the fatal disease; 1 within 3 months, the remainder within 2 to 3 years. It is interesting to note that the livers of these patients presented no scarring to indicate previous injury; this observation is in harmony with our earlier observations.<sup>25</sup>

*Jaundice.* In contrast to the predominant subacute form of the 1942 epidemic, icterus was light or moderate, and rarely deep even during the terminal stage. At least 2 of the fulminant cases remained completely free from jaundice, and in a third only the scleras were faintly tinged. The clinical abstracts of these anicteric cases are included among those chosen to give a representative picture of the disease as seen in individual cases.

*Icterus Index.* The values of the index usually conformed with the relatively light icterus. The data for 60 cases, based upon the highest values recorded, are given in Table VI. The index in approximately two-thirds of the series did not exceed 100. Values above 150 were found in only 8 per cent.

*Total and Differential Leukocyte Count.* During the prodromal period a tendency toward leukopenia was evident in 20 of 27 cases

(Table VII). The differential count disclosed a slight lymphocytosis, *i.e.*, 9 of 10 patients had a lymphocyte-monocyte count between 40 and 49 per cent. In the icteric phase total counts exceeded 11,000 in approximately one-half, the elevation probably resulting from pulmonary complications or hemorrhages. A slight neutrocytosis was also observed during this phase.

*Plasma Proteins.* Determinations made in 25 cases were slightly or definitely below the normal in almost every instance (Table VIII).

TABLE VII  
*Leukocyte Count in Cases of Fulminant Hepatitis*

Leukocyte count	Pre-icteric period	Icteric period
Below—5000	7	0
5000 —6900	13	7
7000 —8900	4	7
9000 —10,900	0	8
11,000—12,900	2	10
13,000—14,900	0	4
15,000 and over	1	14
Total no. of cases	27	50

Because of therapeutic transfusions of blood or plasma, the value of these figures is questionable; without treatment much lower levels might have been obtained.

*Urine.* The urine was usually normal in the first day or two of illness, but as a rule by the third or fourth day changes were noted. The specific gravity was moderately elevated, bile was almost invariably present, albumin of slight degree appeared in two-thirds, and casts in approximately one-half. The casts were sometimes hyaline, sometimes granular, and frequently of both types. Leucine and tyrosine crystals were frequently searched for, but very rarely found.

*Nonprotein Nitrogen; Blood Urea Nitrogen.* Of 31 representative cases selected solely on the basis of completeness of records, the nonprotein nitrogen level of the blood fell within the normal range of 25 to 40 mg. per cent in 16 cases (Table IX). In 15 cases there was evidence of nitrogen retention. In 11 of these, the values ranged between 41 and 60 mg. per cent; in 4 cases they were above 70 mg. per cent.

Blood urea nitrogen was also determined in 10 instances. In 2 the values were 16 and 16.6 mg. per cent, respectively, and the ratio of

TABLE VIII  
*Serum Proteins in 25 Cases of Fulminant Hepatitis*

Below 5.0 gm./100 cc.	3
5.0 —5.9 gm./100 cc.	5
6.0 —6.9 gm./100 cc.	9
7.0 and over	8

blood urea nitrogen to nonprotein nitrogen was approximately normal, *i.e.*, 1:2. In the remaining 8 the urea nitrogen levels were subnormal, ranging from 4 to 12.3 mg. per cent and the blood urea nitrogen/non-

TABLE IX

*Nonprotein Nitrogen and Urea Nitrogen in Representative Cases of Epidemic Hepatitis in Which the Duration of Disease Ranged from 3 to 12 Days*

Case no.	Onset	Death	Date of examination	Nonprotein nitrogen (mg./100 cc. of blood)	Blood urea nitrogen (mg. per 100 cc. of blood)
104032	12/3/43	12/12	1/10	40	
107351	3/16/44	3/20	3/19	55	
			3/20	72	
114638	3/10/44	3/15	3/14	27	
114947	6/3/44	6/12	6/12	60	
119114	9/1/44	9/8	9/7	35	
119183	7/22/44	7/31	7/26	40	
122399	9/5/44	9/9	9/8	50	
			9/9	48	
122680	11/29/44	12/5	12/4	42	
124566	10/10/44	10/15	10/14	43	
125602	7/14/44	7/23	7/22	48	
126048	9/9/44	9/12	9/11	56	
126049	9/7/44	9/12	9/12	50.5	12.3
126108	9/14/44	9/22	9/19	44.4	
			9/21	45.6	
126811	1/9/45	1/13	1/12	36.7	
128003	1/18/45	1/29	1/25	31	16
128072	1/7/45	1/18	1/15	32	4
128558	10/28/44	11/9	11/7	52	
128577	10/28/44	11/5	11/5	60	
128713	11/7/44	11/14	11/11	43	
			11/13	39	5.9
128714	11/11/44	11/16	11/14	36.5	
			11/15	35	
			11/16	35	5.6
129240	1/23/45	2/1	1/25	33	6.2
133205	1/19/45	1/30	1/28	72	
133183	7/30/44	8/4	8/3	103	
131875	12/8/44	12/19	12/19	20	6.2
133190	9/27/44	10/1	9/30	75	
133196	4/11/44	4/20	4/18	40	
133199	1/6/45	1/18	1/16	35	16.6
133201	12/5/44	12/11	12/10	27	5.4
133204	12/27/44	1/5	12/30	40	
131874	9/10/44	9/14	9/12	30	
131667	10/24/44	10/31	10/27	42.8	8.2

protein nitrogen ratios correspondingly varied from 1:3.1 to 1:8. The subnormal levels, both absolute and relative, clearly indicate decreased urea formation. As this substance is formed solely in the liver,<sup>38,39</sup> a decrease in blood concentration is important evidence of hepatic inefficiency.

In the group studied no relation was apparent between duration of disease and nitrogen retention.

*Blood Sugar.* In destructive disease of the liver the blood sugar would be expected to fall to a low level.<sup>30</sup> In most cases of this series such a fall was obscured by the therapeutic administration of glucose. There were, however, 16 cases in which low values were obtained (Table X). In these cases the determinations were made at a time when the patients exhibited central nervous manifestations, *i.e.*, when they were in the terminal stage. The values ranged from 35 to 73 mg. per cent; in 9 they were below, in the remainder above, 55 mg. per cent.

## ILLUSTRATIVE CASES

Pertinent clinical data and laboratory findings are given for 21 representative cases, in which the clinical duration of the disease ranged from 1 to 11 days.

TABLE X

*Low Blood Sugar in Cases of Fulminant Hepatitis in Which the Duration of the Disease Ranged from 3 to 11 Days*

Case no.	Onset	Death	Date of examination	Blood sugar (mg. per 100 cc. of blood)	Remarks (date of examination)
104274	5/16/44	5/23	5/22	38	Drowsy
114638	3/10/44	3/15	3/15	72	Comatose, convulsions
119114	9/1/44	9/8	9/7	37	Alternating restlessness and stupor
122680	11/29/44	12/5	12/4	40	Comatose
122399	9/5/44	9/9	9/9	40	Shock
119183	7/22/44	7/31	7/25	51	Stuporous
124971	10/22/44	10/29	10/26	62	Disoriented, delirious
125482	11/19/44	11/30	11/28	59	Irrational
126048	9/9/44	9/12	9/11	40	Semicomatose, shock
126049	9/7/44	9/12	9/12	35	Comatose, opisthotonos, shock
127334	12/11/44	12/15	12/14	73	Restless
128003	1/18/45	1/29	1/26	63	Semicomatose, convulsions
133205	1/19/45	1/30	1/28	56	Comatose
133182	7/22/44	7/31	7/25	51	Comatose
141771	9/18/44	9/21	9/20	66	Comatose
133190	9/27/44	10/1	10/1	50	Comatose, convulsions

In cases 1 to 10 the predominant manifestations at the onset were those common to a variety of acute infections. Some patients of this group presented serious diagnostic problems; thus, in cases 1, 7, and 10, malaria was suspected. In the second group of cases, 11 to 16, the clinical course of hepatitis was of the more familiar type, being ushered in by gastrointestinal symptoms. A third group (cases 17 to 19) represented the mixed infectious and gastrointestinal form. Case 20 is an example of recurrent hepatitis. In case 21 the first manifestation of the disease was mental disturbance.

In the examples given, 2 patients (cases 2 and 5) remained free from jaundice throughout the course of the disease; in one other (case 7) only the scleras became faintly icteric. In 3 patients (cases 4, 6, and 7) the icterus index did not exceed 35. Six patients (cases 2, 4, 5, 6, 11, and 16) passed into a state of shock. In 5 cases (nos. 2, 4, 6, 8, 10, and 12) the blood sugar sank to low levels. In one case (no. 6) cerebral manifestations led to exploratory craniotomy.

Approximately one-half of these patients had sustained combat trauma and presumably received transfusions of plasma or blood; one patient, not wounded (case 15), had received multiple transfusions of plasma for the treatment of hypoproteinemia. It will be noted that the clinical features of hepatitis were the same in patients who had previously been wounded and received transfusions as in those not wounded, *i.e.*, who had the "naturally" occurring form of hepatitis.

### Case 1

The patient (Army Institute of Pathology, 124057) was a white soldier, 22 years of age.

*Clinical Course.* On April 2, 1944, he complained of generalized aching, occipital headache, fever, nausea, and several bouts of vomiting. His temperature was 102.4° F.; later in the day it declined to 99.8° F.; pulse was 76; respirations, 18. There was rigidity of the voluntary muscles in the right upper quadrant and tenderness over the liver. Icterus became evident on April 4; the patient was restless, hyperactive, had severe pain in the upper abdomen, was persistently nauseated, and vomited repeatedly. His temperature was 96.4° F.; pulse, 90; respirations, 20. On April 5, he became deeply comatose. His pupils were moderately enlarged; there was left external strabismus. Abundant coffee-ground material oozed from mouth and nostrils. The liver was soft and its edge palpable one fingerbreadth below the costal margin. The pulse was of good quality. An algid type of malaria with secondary hepatitis was suspected, but no parasites were found on repeated examination of the blood. During the day the patient's temperature rose to 104.4° F., pulse to 152, respirations to 32; blood pressure dropped from 144/80 to 94/74 mm. Hg. Death occurred on April 5; duration of disease, 3 days.

*Laboratory Findings.* (April 2.) *Blood:* red blood cells, 4.9 million; white blood cells, 4,700, with 51 per cent polymorphonuclear leukocytes and 49 per cent lymphocytes and monocytes. *Urine:* specific gravity, 1030; 1 plus albumin. (April 4.) *Urine:* 1 plus bile. *Icterus index:* 54.

(For illustrations of lesions see Figs. 9, 10, 12, and 19.)

### Case 2

The patient (Army Institute of Pathology, 126048) was a white soldier, 34 years of age.

*Clinical Course.* On July 29, 1944, he was wounded in action by shell fragments, suffering compound comminuted fractures of the left radius, and penetrating wounds of the left thigh and eye. Details of treatment other than debridement were not recorded. He improved steadily and was convalescing at a normal rate. On September 9 the patient felt chilly, had a fever of 103° F., and was nauseated. Examination of the throat, chest, abdomen, and nervous system revealed nothing of note. He felt "stronger" on September 10, but complained of headache and

appeared to be dull and apathetic; temperature was  $100.8^{\circ}$  F. He vomited a dark brown fluid on three occasions. Physical examination was still negative. On September 11 a sudden change took place; the abdomen became rigid; rectal temperature fell to  $94^{\circ}$  F.; pulse was rapid; respirations were intermittent and averaged from 40 to 60 per minute. Chest examination was negative. During the day the patient became semicomatose. The blood pressure dropped to 80/40 mm. Hg. The neck was not rigid; the eyes were open, the pupils widely dilated; the left eye was fixed to light, the right reacted slowly. The patient was treated for shock and the next day seemed slightly improved, with a temperature of  $99^{\circ}$  F., a pulse of 120, and respirations 40 and regular. During the day he became restless. Respirations became shallow and averaged 52 per minute. The temperature rose to  $102^{\circ}$  F. Jaundice did not develop during the course of the illness; the scleras remained clear. Death occurred on September 12; duration of disease, 3 days.

*Laboratory Findings.* (September 11.) *Blood:* red blood cells, 4.5 million; white blood cells, 11,750, with 72 per cent polymorphonuclear leukocytes and 28 per cent lymphocytes; nonprotein nitrogen, 56 mg. per cent; blood sugar, 40 mg. per cent. *Spinal fluid:* protein, 30 mg. per cent; sugar, 28.5 mg. per cent; white blood cells, 9,000; globulin, negative; color, light straw yellow.

(For illustrations of lesions see Figs. 3, 17, 18, and 23.)

### Case 3

The patient (Army Institute of Pathology, 133177) was a white soldier, 31 years, of age.

*Clinical Course.* On June 16, 1944, he complained of severe backache, headache, and intermittent chills. Temperature was  $101.6^{\circ}$  F.; pulse, 88; respirations, 20. On June 17 he experienced severe nausea with occasional vomiting. The liver was enlarged to 2 fingersbreadth below the costal margin, and was moderately tender. His scleras were icteric. On June 18 the temperature had dropped below normal; vomiting was persistent. That night the patient was extremely restless; he hiccupped and vomited repeatedly. On June 19 he became stuporous, tossed about, and failed to respond to questions. Pupils were contracted; the remainder of the physical examination was negative; blood pressure was 110/80 mm. Hg. The patient sank into coma; pulmonary edema developed. Death occurred on June 20; duration of disease, 4 days.

*Laboratory Findings.* (June 17.) *Icterus index*, 65. *Urine:* 2 plus albumin; 2 plus bile. (June 19.) *Urine:* 1 plus albumin; 2 plus bile.

### Case 4

The patient (Army Institute of Pathology, 122399) was a white soldier, 30 years of age.

*Clinical Course.* On June 26, 1944, he was wounded in action by a mortar shell, suffering multiple perforating wounds of bones and chest, and compound fractures of the right humerus and left tibia. Treatment consisted of debridement, plasma, sulfathiazine, and penicillin. On July 4 the left leg was amputated; healing of wounds progressed satisfactorily.

On September 5 he had a chill and a fever of  $103.4^{\circ}$  F.; he complained of headache and diarrhea. Jaundice developed on either September 5 or 6. The patient seemed improved during the day of September 7, but he sweated profusely that night, and on September 8 suddenly went into shock from which he failed to rally despite transfusions of plasma and other treatment. The skin was cold, the pulse thready, and the blood pressure not measurable. The temperature fell to  $97.4^{\circ}$  F.; later in the day, however, it rose to  $102^{\circ}$  and the blood pressure to 100/70 mm. Hg; respirations became shallow and rapid. Death occurred on September 9; duration of disease, 4 days.

*Chemical Examination of the Blood*

Date	Icterus index	Nonprotein nitrogen (mg. per cent)	Sugar (mg. per cent)
Sept. 8	20	50	133
Sept. 9	33	48	40

*Blood Counts*

Date	Red blood cells (millions)	White blood cells	Polymorphonuclear leukocytes (per cent)	Lymphocytes and monocytes (per cent)
Sept. 6	4.2	5,000	52	48
Sept. 7		6,500		
Sept. 8	4.7	13,000	54	45

*Urine*

Sept. 6	Albumin, negative
Sept. 8	Albumin, trace
Sept. 9	Albumin, ++

*Agglutinations*

Sept. 7	Heterophil, negative; typhoid "O," negative Typhoid "H," negative; <i>Leptospira icterohaemorrhagiae</i> : negative
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*Case 5*

The patient (Army Institute of Pathology, 126423) was a white soldier, 25 years of age.

*Clinical Course.* He was wounded in action by a rifle bullet on July 25, 1944. Laparotomy the following day revealed a laceration of the posterior wall of the bladder, multiple lacerations of the ileum, a laceration of the sigmoid, and the peritoneal cavity full of blood and urine. Transfusions, plasma, penicillin, and sulfadiazine were given and by August 5 he was strong enough to be transferred to a general hospital. On arrival he was found to have a functioning colostomy, a suprapubic cystotomy, a urinary fistula through the right buttock, and a pleural effusion. The effusion cleared spontaneously, and despite an episode of thrombophlebitis in the left leg it was possible to deal surgically with his fistulas. Improvement was gradual but steady, and he was evacuated to the United States on October 13. On October 14 his temperature suddenly rose to 104° F. Physical examination and roentgenologic examination of the chest and abdomen were negative. The white blood cell count was 10,150. At 9:30 a.m. on October 15 he vomited; by 1:00 p.m. he was restless, with signs of impending shock. He began to vomit coffee-ground material and passed tarry stools. His white count rose to 19,000, with 72 per cent polymorphonuclear leukocytes and 28 per cent lymphocytes. At 4:15 a.m. on October 16 he began to cough up blood-tinged sputum. Blood pressure was 120/84 mm. Hg; pulse, 116; respirations, 14. At 6:25 a.m. he had a convulsive seizure and went into profound shock. Jaundice did not develop during the course of the illness. Death occurred on October 17; duration of disease, 4 days.

(The cut surface of the liver is shown in Fig. 4.)

*Case 6*

The patient (Army Institute of Pathology, 126049) was a white soldier, 19 years of age.

*Clinical Course.* On July 18, 1944, he was wounded in action, receiving multiple

bullet wounds of the right upper arm which produced a severe compound comminuted fracture of the humerus. Secondary closure of the wounds was done and he convalesced rapidly. On September 7 generalized aching developed and his temperature rose to  $102.8^{\circ}$  F. On September 8 he complained of nausea; on September 9 his temperature was  $101^{\circ}$  F. General physical examination was negative, and when the cast was removed in order to inspect the wounds, they were found to be healing satisfactorily. On September 11, although the temperature was normal, nausea and vomiting persisted. The patient fell out of bed during the night and afterward seemed disoriented, although physical examination was still negative. The following day, September 12, jaundice was evident. Irrationality was rapidly followed by deep coma, interrupted by repeated convulsive seizures, some of which were associated with opisthotonos. The pupils were equal; no papilledema was present. There were bilateral positive Babinski signs, and bilateral sustained ankle clonus; abdominal and cremasteric reflexes were absent, as were Chvostek's and Trousseau's signs. It was thought that a subdural hematoma might have resulted from the fall out of bed; however, cranial exploration showed no evidence of intracranial bleeding and the ventriculogram was normal. Blood pressure fell during the operation from 190/100 to 70/55 mm. Hg. Death occurred on September 12; duration of disease, 5 days.

*Laboratory Findings.* (September 11.) *Blood:* white blood cells, 8,250. (September 12.) Chemical examination: nonprotein nitrogen, 50.5 mg. per cent; urea nitrogen, 12.3 mg. per cent; sugar, 35 mg. per cent; icterus index, 35. *Spinal fluid:* total protein, 20 mg. per cent; cells, 22 (16 polymorphonuclear leukocytes and 6 lymphocytes and monocytes). *Urine:* color, dark amber; specific gravity, 1.027; albumin, 3 plus; sugar, negative; acetone, 1 plus; diacetic acid, negative.

### Case 7

The patient (Army Institute of Pathology, 133183) was a white soldier, 26 years of age.

*Clinical Course.* On July 30, 1944, he complained of chills, fever, headache, and general aching which came on suddenly and persisted for several days, accompanied by anorexia, nausea and vomiting. He was admitted to the hospital on August 2 when he appeared acutely ill. Temperature was  $102.6^{\circ}$  F.; pulse, 100; respirations, 20. The only positive finding on physical examination was a palpable and tender spleen. A tentative diagnosis of tertian malarial fever was made; however, repeated blood smears were negative for malarial parasites. There was no improvement during the night and on the morning of August 3 the temperature had risen to  $104.4^{\circ}$  F.; pulse, 96; respirations, 20. The patient complained of headache, and pain in the chest. The icterus index was 28; the blood nonprotein nitrogen, 103 mg. per cent. The physical findings were unchanged. During the day the temperature subsided to  $98^{\circ}$  F. and by the next morning, August 4, to  $96.4^{\circ}$ . He became disoriented and had hallucinations. The liver and spleen were palpable. The lungs appeared clear; there was no roentgenologic evidence of pneumonic consolidation or fluid. The heart was normal. The deep reflexes were hyperactive; the superficial reflexes diminished. During the day the patient gradually became cyanotic. Definite jaundice never developed and only a faint icteric tint was noted in the scleras post-mortem. Death occurred the evening of August 4; duration of disease, 6 days.

### Case 8

The patient (Army Institute of Pathology, 124971) was a white soldier, 24 years of age.

*Clinical Course.* He was wounded in action by an exploding shell on August 13, 1944, sustaining damage to both orbital regions. For several weeks he continued to have a draining orbital sinus; his eyesight was lost; his general physical



condition was good. On October 22 he awakened with a severe throbbing headache and during the day his temperature was between 99° and 100° F. On October 23, it rose to 103.5° F.; he vomited and complained of considerable muscular aching. His skin took on an icteric tint; no other physical findings of significance were noted. Between October 23 and 26 jaundice deepened, and his condition became progressively worse. The temperature remained between 99° and 100° F. He was disoriented. On October 29 he had several generalized convulsions and became deeply comatose. Death occurred on October 29; duration of disease, 7 days.

*Chemical Examination of the Blood*

Date	Icterus index	Sugar (mg. per cent)	Urea nitrogen (mg. per cent)	Serum protein (gm. per cent)	Albumin (gm. per cent)	Globulin (gm. per cent)
Oct. 25	33					
Oct. 26	64	62	9.3	5.2	3.3	1.9

*Blood Counts*

Date	Red blood cells (millions)	White blood cells
Oct. 22	4.0	5,900
Oct. 23		6,500

*Case 9*

The patient (Army Institute of Pathology, 125602) was a white soldier, 30 years of age.

*Clinical Course.* On July 14, 1944, malaise and general aching began and the patient was admitted to the hospital. His temperature was 104° F.; he was not jaundiced; the only significant physical finding was enlargement of the liver. On July 18 a moderate degree of jaundice of the skin and scleras was noted. On July 21 the patient became drowsy and difficult to arouse, later sinking into coma. There was a terminal fever of 106° F. Death occurred on July 21; duration of disease, 7 days.

*Chemical Examination of the Blood*

Date	Icterus index	Nonprotein nitrogen (mg. per cent)	Blood sugar (mg. per cent)
July 19	54		
July 22	108	48	100

*Blood Counts*

Date	Red blood cells (millions)	White blood cells	Polymorphonuclear leukocytes (per cent)	Lymphocytes and monocytes (per cent)
July 19	5.12	5050		
July 21		10400	57	43

*Urine*

Date	Albumin	Bile
July 19	++	
July 21	++	+

(For photomicrograph of kidney see Fig. 30.)

## Case 10

The patient (Army Institute of Pathology, 133205) was a white male, 25 years of age.

*Clinical Course.* On January 19 his illness was ushered in with ocular headache, general aching, chills, fever, loss of appetite, and mild nausea. He was admitted to the dispensary on January 20. Symptoms became progressively worse during the next 3 days, and on January 23 jaundice became evident. The patient was sent to the hospital with a diagnosis of malaria or hepatitis. On admission, temperature was 100.8° F.; pulse, 82; respirations, 16. He complained of headaches, mild abdominal pain, complete loss of appetite, occasional nausea and vomiting. Physical examination revealed moderate jaundice; the liver was slightly enlarged and tender; the spleen also was enlarged and tender; the chest was clear. Neurologic examination was negative. On January 25 the patient appeared slightly improved; he was afebrile, but liver and spleen were very tender, and icterus was deepening. He vomited during the night. On January 27 he was very drowsy but could be aroused easily, was well oriented, looked critically ill, and had vomited two or three times. By January 28 the patient was deeply jaundiced; he was in coma but responded to painful stimuli. The liver was palpable two fingersbreadth below the costal margin. On January 29 the coma deepened; there was some rigidity of the extremities; pulmonary edema developed. Death occurred on January 30; duration of disease, 11 days.

*Chemical Examination of the Blood*

Date	Nonprotein nitrogen (mg. per cent)	Blood sugar (mg. per cent)	Icterus index
Jan. 27			133
Jan. 28	72	56	124

*Blood Counts*

Date	Red blood cells (millions)	White blood cells	Polymorphonu- clear leukocytes (per cent)	Lymphocytes and monocytes (per cent)
Jan. 24	4.9	9,000	74	26
Jan. 27	4.7	8,000	71	29
Jan. 29	4.5	10,000	76	24

*Blood Examinations for Malarial Parasites*

Jan. 24, 25, 26, 27, 28, and 29 Negative

## Case 11

The patient (Army Institute of Pathology, 126811) was a white soldier, 31 years of age.

*Clinical Course.* On October 3 he received a perforating gunshot wound of the abdomen necessitating laparotomy and colostomy. He made a good recovery, gained 10 pounds, and felt well for the next 3 months. On December 28 the colostomy was closed under local anesthesia; the postoperative course was uneventful. On January 9 he complained of pain in the epigastrium, became nauseated and vomited. On January 11 he was still slightly nauseated; the abdomen was soft and flat; the lungs were clear. On January 12 temperature mounted to 104° F.; the patient vomited and was nauseated; by evening he was somewhat disoriented. The conjunctiva was definitely icteric; the urine was dark; the stools light brown; the abdomen was soft and tender over both the hepatic and splenic regions, but the liver was not palpable. On January 13 the patient suddenly collapsed; the

pulse became very rapid and almost imperceptible; respiration was strained as in marked air hunger; blood pressure fell to 84/66 mm. Hg; temperature was 103.2° F. by rectum. The patient was alternately comatose and awake and restless. Blood pressure measurements were made repeatedly and, although they could not be accurately obtained, approximated 88/60 mm. Hg. The pulse became very thready and weak, the breathing shallow. Death occurred on January 13; duration of disease, 4 days.

*Laboratory Findings. Chemical examination of the blood:* Nonprotein nitrogen (January 12), 37 mg. per cent; icterus index (January 13), 60. *Urine* (January 12): albumin, 2 plus; bile, 0.

(For photomicrographs of kidney see Figs. 31 and 32.)

### Case 12

The patient (Army Institute of Pathology, 128714) was a white soldier, 28 years of age.

*Clinical Course.* On January 9, 1944, he suffered severe burns on the legs, when gasoline spilled on his uniform and was ignited. The resulting burns were debrided, sulfadiazine ointment was applied, and he received plasma intravenously. On October 25 multiple grafts were applied to the wound and by November 5 they had taken. On November 11 the patient complained of abdominal fullness and distress. On November 13 he vomited, and also noticed that his skin had become yellow and his urine dark. On November 14 he vomited a great deal but had no other complaints. On November 15 his condition appeared good; blood pressure was 120/80 mm. Hg. He took small amounts of fluid eagerly and retained them. In the afternoon, however, he became increasingly restless and later delirious. Respirations were labored and pulse weak. Edema of the lungs was noted. Death occurred on November 16; duration of disease, 5 days.

#### Chemical Examination of the Blood

Date	Nonprotein nitrogen (mg. per cent)	Urea nitrogen (mg. per cent)	Icterus index	Blood sugar (mg. per cent)
Nov. 14	36		77	
Nov. 15	35			111
Nov. 16 (a.m.)	35	5.6		97
Nov. 16 (p.m.)	44	5.5	175	44

#### Blood Counts

Date	Red blood cells (millions)	White blood cells	Polymorphonu- clear leukocytes (per cent)	Lymphocytes and monocytes (per cent)
Nov. 14	5.3	8,350	72	28
Nov. 16	5.2	16,450	90	10

### Case 13

The patient (Army Institute of Pathology, 114947) was a Javanese soldier of the Dutch Army, 31 years of age.

*Clinical Course.* On or about June 3 he noticed vague abdominal pain and began to vomit. His stools became light in color. When he was admitted to the hospital on June 9 the temperature was normal, liver and spleen were neither palpable nor tender, and the abdomen was soft. The scleras were then normal,

but on June 10 became slightly icteric. The stools were clay-colored. The patient vomited coffee-ground material. On June 11 he became irrational, over-active, and then listless. On June 12 scleras were definitely icteric; the patient sank into coma. For the first time since the onset of symptoms there was fever, which rose to 104° F. Death occurred on June 12; duration of disease, 9 days.

*Chemical Examination of the Blood*

Date	Icterus index	Nonprotein nitrogen (mg. per cent)
June 11	85	
June 12	160	60

(For photomicrograph of liver see Fig. 11.)

*Case 14*

The patient (Army Institute of Pathology, 104032) was a white soldier, 22 years of age.

*Clinical Course.* On December 3, 1943, he complained of nausea and vomiting. He was admitted to the hospital on December 4, appearing neither acutely ill nor in discomfort; nausea and vomiting were not severe; there was slight tenderness in the upper abdomen. Temperature was 99° F.; pulse, 88; respirations, 22; blood pressure, 100/80 mm. Hg. On December 7 an icteric tint to the scleras was noticeable. During the next 2 days jaundice deepened rapidly and became intense by December 10. The liver edge was palpable. The patient became irrational, later comatose. Death occurred on January 12; duration of disease, 9 days.

*Chemical Examination of the Blood*

Date	Icterus index	Nonprotein nitrogen (mg. per cent)
Dec. 8	25	
Dec. 10		40
Dec. 11	75	

*Blood Counts*

Date	Red blood cells (millions)	White blood cells	Polymorphonu- clear leukocytes (per cent)	Lymphocytes and monocytes (per cent)
Dec. 4	4.3	6,350	76	24
Dec. 5	4.3	6,000	60	40
Dec. 8	4.1	6,850	77	23
Dec. 10	4.8	5,100	79	21

*Urine*

Date	Bile
Dec. 4	0
Dec. 8	+
Dec. 10	+

*Case 15*

The patient (Army Institute of Pathology, 111844) was a white soldier, 22 years of age.

*Clinical Course.* He was admitted to the hospital in January, 1944, with edema of the hands and feet. Hypoproteinemia was found, without alteration of the albumin-globulin ratio. The origin of the protein deficiency was obscure; all tests for liver function were normal. He was placed on a suitable diet and given five plasma transfusions. Within 4 weeks his blood proteins had reached normal levels and he was discharged. On April 5 he complained of abdominal pain and nausea, and jaundice appeared. When the patient was admitted to the hospital on April 12 the liver was enlarged and tender and the urine dark brown. His condition deteriorated rapidly. On April 14 he was delirious, his abdomen was distended, and jaundice was deepening; coma ensued. The clinical course in the hospital was entirely afebrile. Death occurred on April 15; duration of disease, 10 days.

#### *Chemical Examination of the Blood*

Date	Icterus index	Urea nitrogen (mg. per cent)	Serum protein (gm. per cent)	Albumin (gm. per cent)	Globulin (gm. per cent)
April 12	60				
April 14	105	12	6.9	3.0	3.9
April 15	105	14	7.0	3.2	3.8

#### *Blood Counts*

Date	Red blood cells (millions)	White blood cells	Polymorphonu- clear leukocytes (per cent)	Lymphocytes and monocytes (per cent)
April 12		7,500	63	37
April 14		9,150	70	30

(See Fig. 26 for photomicrograph of proliferating bile ducts.)

#### *Case 16*

The patient (Army Institute of Pathology, 128072) was a white soldier, 26 years of age.

*Clinical Course.* On October 23, 1944, he was wounded in action by an enemy mine, sustaining traumatic amputation of the lower part of the left leg. Early treatment included administration of sulfadiazine, penicillin, and transfusions of whole blood and blood plasma. Convalescence was uneventful; the soldier was returned to the United States and under further treatment the stump healed satisfactorily. On January 7, 1945, he complained of vague abdominal discomfort, loss of appetite, aversion to greasy foods, general malaise, nausea and vomiting. During the next few days, loss of appetite and abdominal discomfort continued, but nausea and vomiting subsided. On January 13 he had a mild attack of urticaria and became slightly jaundiced. The liver was not definitely palpable, but was slightly tender and increased in size to percussion. Diagnosis of acute hepatitis was made and treatment instituted. The patient's condition remained essentially unchanged until the morning of January 17, when he suddenly became confused, then disoriented, and finally stuporous and comatose. Oozing of bloody fluid from the mouth became profuse. Icterus deepened in intensity; the liver, which 3 days before had been somewhat enlarged, now was found to be definitely smaller to percussion. The blood pressure fell from 155/90 to 84/40 mm. Hg. There was a terminal rise of temperature to 105° F. Death occurred on January 18; duration of disease, 11 days.

*Laboratory Findings.* (January 15.) *Blood:* icterus index, 76; white blood cells, 10,400; (January 17) blood nonprotein nitrogen, 32 mg. per cent; blood urea nitrogen, 4 mg. per cent.

## Case 17

The patient (Army Institute of Pathology, 129051) was a white soldier, 27 years of age.

*Clinical Course.* On August 7, 1944, he was wounded in action by a rifle bullet, sustaining a compound fracture of the left os calcis. A suppurating sinus persisted, and he was evacuated to the United States. Between November 15 and December 22 he was treated with penicillin. At Christmas he was well enough to receive a convalescent furlough. On his return, penicillin therapy was again instituted. Although evidence of healing of the bone defect could be seen in the roentgenogram, a sinus still persisted which required surgical treatment. Before the contemplated operation the patient was granted another furlough. On February 14, upon arriving at the home of his sister, he stated that he was not feeling well and complained of pain in the right hip, nausea and vomiting. It was noted that his scleras were yellowish and his skin slightly jaundiced. He declined to return to the hospital as he did not believe himself seriously ill. On February 15 he stayed overnight with a friend and on the morning of February 16 he was restless and at times irrational. He was taken by ambulance to an Army hospital, arriving on the morning of February 18. On admission he was semiconscious. His pupils were unequal, the left being larger than the right; both reacted to light. His neck was definitely rigid. Spinal puncture was done; the fluid was clear and apparently not under increased pressure. Physical examination disclosed slight icterus and hyperactive reflexes. The abdomen was soft and the lower extremities were spastic. Râles were heard over the chest. The diagnosis of meningitis was entertained although examination of the spinal fluid was not confirmatory. Pulmonary edema developed. Death occurred on February 18; duration of disease, 4 days.

(For photomicrograph of liver see Fig. 16.)

## Case 18

The patient (Army Institute of Pathology, 128329) was a white soldier, 23 years of age.

*Clinical Course.* On May 26 anorexia, nausea, and fever developed, and he was admitted to the hospital on May 27. He appeared acutely ill; temperature was 100°, later 103° F. The spleen and liver were not palpable or tender. Chest examination was negative. On May 28 his general condition was improved; temperature dropped to 99.2° F., and subsequently remained normal. The patient was eating well. On May 31 jaundice was noted for the first time; he also complained of nausea. On June 1 he appeared ill, with lassitude, nausea and vomiting; jaundice deepened rapidly. On June 2 he became semistuporous and restless; there was slight tenderness in the upper abdomen. On June 3 profound coma set in, but there were no abnormal neurologic findings. Pulmonary edema developed. Death occurred on June 4; duration of disease, 9 days.

*Chemical Examination of the Blood*

Date	Icterus index	Urea nitrogen (mg. per cent)	Serum protein (mg. per cent)	Albumin (gm. per cent)	Globulin (gm. per cent)
May 31	65	5.7	5.8	3.5	2.3

*Urine*

Date	Albumin	Bile
May 28		
May 31	++	o
June 2		+
June 4	++	+

## Case 19

The patient (Army Institute of Pathology, 129240) was a white soldier, 27 years of age.

*Clinical Course.* On October 23, 1944, he was wounded in action, suffering traumatic amputation of the left foot. He was treated by debridement, transfusions of plasma and whole blood, and penicillin. Convalescence was uneventful until January 23, 1945, when anorexia and nausea appeared and he noticed that his urine was becoming darker and his feces lighter than normal. Sensations of chilliness and general malaise were followed by a rise in temperature to 102° F. On January 24 he had a second chill; his temperature now rose to 104° F. He was jaundiced. The liver edge extended 2 fingersbreadth below the costal margin and was not tender. The spleen was easily palpable. On January 26 temperature fell to normal and remained between 97° and 99° F. for the next few days; nausea diminished; his appetite, however, remained very poor. On January 28 the patient suddenly became much worse; he vomited repeatedly; jaundice increased in intensity; the liver appeared to diminish in size; he became mentally confused and delirious; bled from the mouth and nose; and lapsed into coma. Death occurred on February 1; duration of disease, 9 days.

*Chemical Examination of the Blood*

Date	Icterus index	Nonprotein nitrogen (mg. per cent)	Serum protein (gm. per cent)
Jan. 25	33	36	6.2
Jan. 28	120		

*Blood Counts*

Date	Red blood cells (millions)	White blood cells	Polymorphonuclear leukocytes (per cent)	Lymphocytes and monocytes (per cent)
Jan. 25	4.5	5560	60	40

Repeated examinations of blood for malarial parasites were negative.

## Case 20

The patient (Army Institute of Pathology, 133194) was a white soldier, 21 years of age.

*Clinical Course.* He had been hospitalized for "hepatitis without jaundice," once for 2 months, beginning April, 1944, and a second time for 4 months, beginning June 20, 1944. His symptoms consisted chiefly of distress in the upper abdomen radiating to the back, anorexia, and intolerance for greasy foods. He was also thought to be psychoneurotic with seclusiveness and dependency; a diagnosis of "anxiety state" had been made. On the third entry to the hospital, on November 6, physical examination was negative; liver and spleen were not palpable. Laboratory studies the week after admission were negative; icterus index, 8; cephalin flocculation, 0/0; blood phosphatase, 4.4 units. Hemoglobin, 85 per cent; red blood cells, 4,500,000; white cells, 5,250, with 62 per cent polymorphonuclear leukocytes and 38 per cent lymphocytes. He complained of constant upper abdominal pain throughout the several weeks he was observed as a psychoneurotic patient. On November 22 he complained of headache, anorexia, and nausea. On November 23 he felt feverish; temperature rose to 100° F.; abdominal pain became more severe. Physical examination showed tenderness but no rigidity in the right upper quadrant. That evening he had a chill lasting 30 minutes. The morning urine,

November 24, was dark; the scleras were questionably icteric. Tenderness in the right upper quadrant persisted, but the liver could not be felt. By November 28 the scleras were definitely icteric and the icterus index was 27. He became acutely ill with severe vomiting. The right upper quadrant was exquisitely tender. On November 30 restlessness alternated with lethargy, vomiting continued, and the patient went into coma. Death occurred on December 1; duration of disease, uncertain (terminal illness, 8 days).

### Case 21

The patient (Army Institute of Pathology, 123465) was a white soldier, 25 years of age.

*Clinical Course.* On July 30, 1944, he was admitted to a clearing company in a state of mental agitation. The diagnosis was "battle fatigue." While on the ward he became disturbed, fearing Japs were going to attack him. He had no other complaints and physical examination was negative except for evidence of recent loss of weight. There was no jaundice. Under mild sedation he became somewhat quieter. In the evening of the same day his respiration ceased suddenly and no pulse was perceptible. Death was thought to have occurred instantly, due either to cardiac stoppage as a result of vagus stimulation secondary to fright, or to pulmonary embolism. Death occurred on July 30; duration of disease, uncertain (clinically, 1 day).

(For photomicrographs of liver see Figs. 8, 15, 24, and 27.)

### PATHOLOGIC ANATOMY

In fulminant hepatitis, lesions other than those in the liver are not striking. The changes in the gallbladder, extrahepatic bile ducts, regional lymph nodes, bone marrow, and the hemorrhagic phenomena are of the same general type as described previously. We may limit ourselves, therefore, to accounts of the liver, ascites, spleen, intestines, kidney, and brain.\* Consideration of the prostate will be reserved for a future communication. No noteworthy pathologic changes were found in the other organs.

*Similarity of Lesions of "Spontaneous" and "Inoculation" Hepatitis.* The lesions of the several epidemiologic forms of hepatitis included in this study are in every respect indistinguishable. Hence an account of the pathologic anatomy may be given for the entire series.

### LIVER

Two processes characterize the pathologic picture of the liver in fulminant hepatitis: extreme and often complete destruction of liver cells, and marked inflammatory response. From naked-eye examination of the organ, however, it is not possible to surmise the extensive destruction of the parenchyma or the degree of inflammation. The vague term "acute yellow atrophy," although connoting rapid break-

\* We are grateful to Lt. Col. Philip Custer for examining many sections of spleen, and to Dr. Nathan Malamud and Major Webb Haymaker for advice regarding the changes in the brain.



down of hepatic parenchyma, is not descriptive of the appearance, and cannot properly be applied.

### *Gross Appearance*

In the great majority of cases the liver is reduced in size, but the shrinkage is usually moderate. The weights are given in Table XI: in one-half they range between 1000 and 1400 gm., and in approximately one-third of the series they are below 1000 gm. Excessive reduction to below 800 gm. occurs in only 6 per cent, and, at the other extreme, weights exceed 1600 gm. in 11 per cent. The median for the entire group is 1150 gm. The data upon which Table XI is based are graphically analyzed in Text-Figure 7 (p. 904), first, with respect

TABLE XI  
*Weight of Liver in 101 Cases of Fulminant Hepatitis*

	No. of cases	Associated with ascites
Below 800 grams	6	1
800—999	25	5
1000—1199	28	9
1200—1399	22	6
1400—1599	9	1
1600—1799	8	6
Over 1800	3	2
Total	101	30

to duration of disease; second, with reference to the two main epidemiologic forms (represented in the figure as "wounded" and "not wounded"), and, third, in relation to the presence or absence of ascites. Great scattering of weight and absence of correlation with duration are evident. For example, the livers from the 12 cases having a duration of 4 days weigh from 600 to 1800 gm., with a median of 1200 gm. Livers from cases having durations of only 2 or 3 days present as great shrinkage or as little change in size as those with a longer course. Livers from fatal cases of "naturally" occurring hepatitis do not differ significantly in weight from those of "inoculation" hepatitis. The relation to ascites will be discussed later.

The surface is always either smooth or finely wrinkled (Fig. 1); it is never deformed by the nodular or tumor-like elevations characteristic of the subacute stage. The shape is little altered, except that the anterior edge commonly is sharp. The capsule is usually transparent. Small subcapsular hemorrhages are common. The color is not distinctive and varies through shades of red, purple, and brown; mottling is common and such combinations as gray-red and purple, or tan and red, are often observed. The organ is usually soft and flabby, sometimes to

such an extent that it flattens when laid on the table; but the flabbiness may be masked by such great engorgement that the organ is tense.

The cut surface nearly always shows diffuse involvement, most frequently in the form of an exaggerated "nutmeg" pattern (Figs. 3 to 7). The lobular peripheries are outlined by pale grayish or yellow-gray bands of variable width, which contrast with the dark red or purple of the slightly sunken inner part of the lobules. In other cases, on the contrary, the landmarks are so indistinct that the cut surface resembles that of an acutely congested spleen (Fig. 2). The blood content also varies considerably; in some instances the inner zones of every lobule ooze blood copiously, in others the entire organ is relatively ischemic; sometimes areas of engorgement and relative bloodlessness alternate. The cut surfaces nowhere have a greasy sheen; the bands which outline the lobules have the fresh appearance of healthy tissue; the lobular interiors, when not too greatly congested, are dull grayish red, but not fatty.

In roughly 10 per cent of the cases the changes are most marked in the left lobe, which is more shrunken and has a more wrinkled surface.

### *Microscopic Appearance*

In this section we shall deal principally with the lesions typical of the fulminant form of hepatitis in which the clinical duration of disease is less than 10 days. Later a brief account will be given of the changes found in two other groups of cases, namely, those which survive from 10 to 19 days, and those of unknown clinical duration. These two groups are important because they not only show transition stages to the subacute form previously studied, but they also illustrate the disparity which may exist between the clinical course and the anatomic changes.

As already stated, two processes dominate the microscopic picture of fulminant hepatitis: extensive destruction of liver cells and marked inflammatory reactions. Regenerative hyperplasia of surviving parenchyma, which is so prominent a feature of the subacute form, is lacking or minimal, and is confined to biliary rather than to hepatic epithelium.

*Destruction of Liver Cells.* The destructive process, in the majority of cases, involves all parts of the liver uniformly. As a rule, destruction of liver cells is extensive and, in many instances, complete (Figs. 8, 9, and 15); but often a narrow rim, a few cells in width, persists at the lobular periphery (Fig. 16). Very rarely the destruction instead of being predominantly central is peripheral, sparing small isolated patches of parenchyma in the interior.

In a few of the most rapidly fatal cases a scattering of shadow forms

of liver cells sometimes remain but it is distinctive that the dead cells are removed rapidly. In the entire series no example of the earliest stage in the necrotic process was encountered; cell destruction was always advanced. As a rule, even in cases with a clinical history of only 3 or 4 days no traces of dead cells were found. These facts suggest that the agent bringing about necrosis effects speedy lysis and enzymic digestion rather than cell coagulation as seen in the necrosis of anoxemia and after many hepatic poisons. Less commonly the few remaining cells show degenerative changes, such as small irregular droplets of fat or other vacuoles in the cytoplasm. These changes are regarded as secondary to the profound disturbances of blood supply that accompany necrosis of the parenchyma.

*Persistence of Reticulum and of Sinusoids.* The destructive process specifically affects liver cells; the other components of the lobule escape. The reticulum, in all cases, is intact (Fig. 17). The inter-reticular spaces, formerly occupied by liver cells, in some instances become packed with monocytes and erythrocytes and little shrinkage occurs (Fig. 21); in other instances the meshes are empty and collapsed. The sinusoids usually are widely engorged (Figs. 9 and 22); small hemorrhages occasionally occur. In brief, the liver in fulminant hepatitis is rapidly reduced to a spongy framework, infiltrated by inflammatory cells and often distended with blood.

*Inflammatory Reaction.* In the present series, inflammatory cellular infiltration is considerably more marked than in the subacute form. The cell reaction is most conspicuous at the lobular periphery, *i.e.*, in the portal stroma and the interlobular boundary (the "septum vasculare").<sup>10,19,20</sup> Within the lobular remnants inflammation is less prominent and of somewhat different type.

Often whole lobules are outlined by bands of densely packed cells (Figs. 8 and 9). The composition of the infiltrate varies from case to case; a fairly representative picture is shown in Figure 11. It will be noted that the cells are well preserved and that there is no indication of breakdown. They are chiefly mononuclear forms—reticulo-endothelial derivatives, plasma cells, and lymphocytes—but neutrophils and eosinophils in relatively small numbers are almost invariably present (Fig 13).

Within the lobular remnants, mobilized and proliferated macrophages predominate; frequently they become so large and numerous as to give a spurious appearance of a "parenchyma" consisting of disunited but preserved liver cells (Fig. 14). The macrophages commonly contain a brownish, sudanophilic, and faintly acid-fast pigment, lipofuscin, which probably is derived from the disintegrated hepatic cells.

Lymphocytes, plasma cells, and granulocytes, while not numerous, are present also.

Inclusion bodies, cytoplasmic or intranuclear, such as occur in many different virus infections, are not encountered in any kind of cell.

*Efferent Veins.* In the subacute form of hepatitis of the 1942 series, endophlebitis of the efferent veins was a conspicuous, though not a pathognomonic, feature. In the fulminant form it is less commonly encountered. Frequently, however, the walls of the central lobular and smaller collecting veins are much thickened and of homogenous, hyaline texture (Fig. 18).

*Regeneration of Liver Cells.* In no case of the fulminant group is there any noteworthy degree of regenerative hyperplasia. At most, the persistent cells are large, irregular in outline, and multinucleated; significant increase in number is not apparent. Regeneration obviously cannot take place when destruction of the parenchyma is complete.

*Proliferative Changes in Bile Ducts.* It is of importance to note that in most cases, even the most acute, the small twigs of the bile ducts, both septal (perilobular) and interlobular, exhibit some evidence of proliferation. These little ducts normally are inconspicuous. The degree of proliferation attained is illustrated by representative photomicrographs. In Figure 23 is shown a septal duct composed of large, closely packed cells with prominent nuclei; the duration of disease in this case was only 3 days. In Figure 24 may be seen a more conspicuously proliferating duct lying in the portal stroma; the duration in this case was, clinically, less than 1 day. In 2 cases with duration of 9 or 10 days, the ducts have irregular shapes, due to budding and branching, and the nuclei of the component cells are deeply chromatic (Figs. 25 and 26).

These examples illustrate the rapid proliferation of which biliary epithelium is capable.

*Lesion in Patients Surviving from 10 to 19 Days.* In approximately one-third of the patients surviving 10 to 19 days the lesions were identical with those of the more fulminant cases. Thus, in some instances, the microscopic picture of the liver from patients who survived for 10 or more days was indistinguishable from that when death occurred within 3 or 4 days after onset. More often, however, the lesions were similar to those encountered at corresponding periods of survival in the 1942 series. In agreement with the findings in that series the involvement of the liver in the less fulminant group is not uniform. This difference is shown in Figures 28 and 29, which represent fields from two hepatic areas of a patient who survived for 12 days. Figure 28 shows a region in which destruction of hepatic parenchyma is in-

complete, while Figure 29 is from an area in which destruction is practically complete and the lobular remnants are outlined by proliferating bile ducts.

*Correlation between Gross and Microscopic Changes.* The degree of engorgement and of inflammatory infiltration are clearly correlated with size and appearance of the liver. As to the former, the content of blood accounts for the great variations of weight, despite the fact that in all cases only traces of parenchyma remain. The predominant "nutmeg" pattern of the cut surface is brought about by a combination of the marked inflammatory infiltration at the lobular periphery and the engorgement in the interior of the lobular remnants. In cases where the cut surface resembles that of a congested spleen, engorgement outbalances inflammation.

As has been stated, the gross appearance of the liver gives no indication of the extreme destruction of the parenchyma.

#### *Disparity between Lesions and Clinical Duration*

The clinical duration is not always correlative with the pathologic findings; sometimes the process is obviously older than the history suggests, less often the reverse is true. For example, in many instances no relation can be established between duration of disease and degree of inflammatory reaction; its severity in some of the shortest cases makes the conclusion inescapable that the reaction must have antedated the first symptoms. Similarly, there is often no correspondence to the extent of parenchymatous destruction or to the degree of proliferation of the small bile ducts. For example, the lesions shown in Figures 8, 15, 24, and 27 are from a patient with a clinical course of less than 1 day; but all traces of dead liver cells have been removed and the bile ducts exhibit active proliferation. In numerous other very early cases like pictures are obtained. Again, in some of the most fulminant cases, jaundice appeared on the first day; no doubt disintegration of the liver was advanced before symptoms began.

An even greater discrepancy between clinical history and pathologic changes was noted in a group of 18 cases which purposely were omitted from the analysis of duration given in Tables I, II, IV, and XII. In this group, 5 cases with a short course showed evidence of well marked regeneration with nodularity of the livers. In contrast, 8 cases prolonged for more than 20 days presented the acute changes characteristic of the fulminant form. It is possible that some of the latter were recurrent infections which ran an acute course.

It may be concluded that in the more rapidly fatal cases the lesions are older than the clinical manifestations; in other words, when symp-

toms appear the liver is already definitely involved; in some instances the clinical course may be "silent," or the symptoms minimal even when death occurs within a few days. These conclusions are borne out by the studies of specimens taken for biopsy at different stages of the disease in nonfatal cases; here also the lesions frequently appear older than the clinical duration.<sup>19,40</sup>

### *Ascites*

The incidence of ascites has been tabulated for 140 cases (Table XII). There is a definite correlation between ascites and clinical duration of disease: the incidence rose from 24 per cent when death occurred within 10 days, to 43 per cent when it was delayed from 10

TABLE XII  
*Occurrence of Ascites in 140 Cases of Hepatitis  
(New Series)*

Duration of disease	Total no. of cases	Ascites	Per cent of cases
To 9 days	68	16	24
10-19 days	33	14	43
Over 19 days	39	27	70

to 19 days, and to 70 per cent when the course was more protracted. The amount of fluid in the fulminant cases was usually less than 1 liter, whereas in the others, larger volumes (2 or 3 liters) were the rule.

The relation between ascites, weight of liver, and duration of disease is shown for wounded and nonwounded cases in a distribution graph (Text-Fig. 7). It will be noted that ascites occurred even in the most fulminant cases. The relation to weight of liver is interesting: ascites was more common when the liver was but little shrunken or was actually enlarged, than when its bulk was considerably decreased. Thus, in the 25 cases with livers weighing above 1300 gm. ascites was observed 11 times, whereas it occurred only 4 times in the 25 cases having the smallest livers (below 920 gm.).

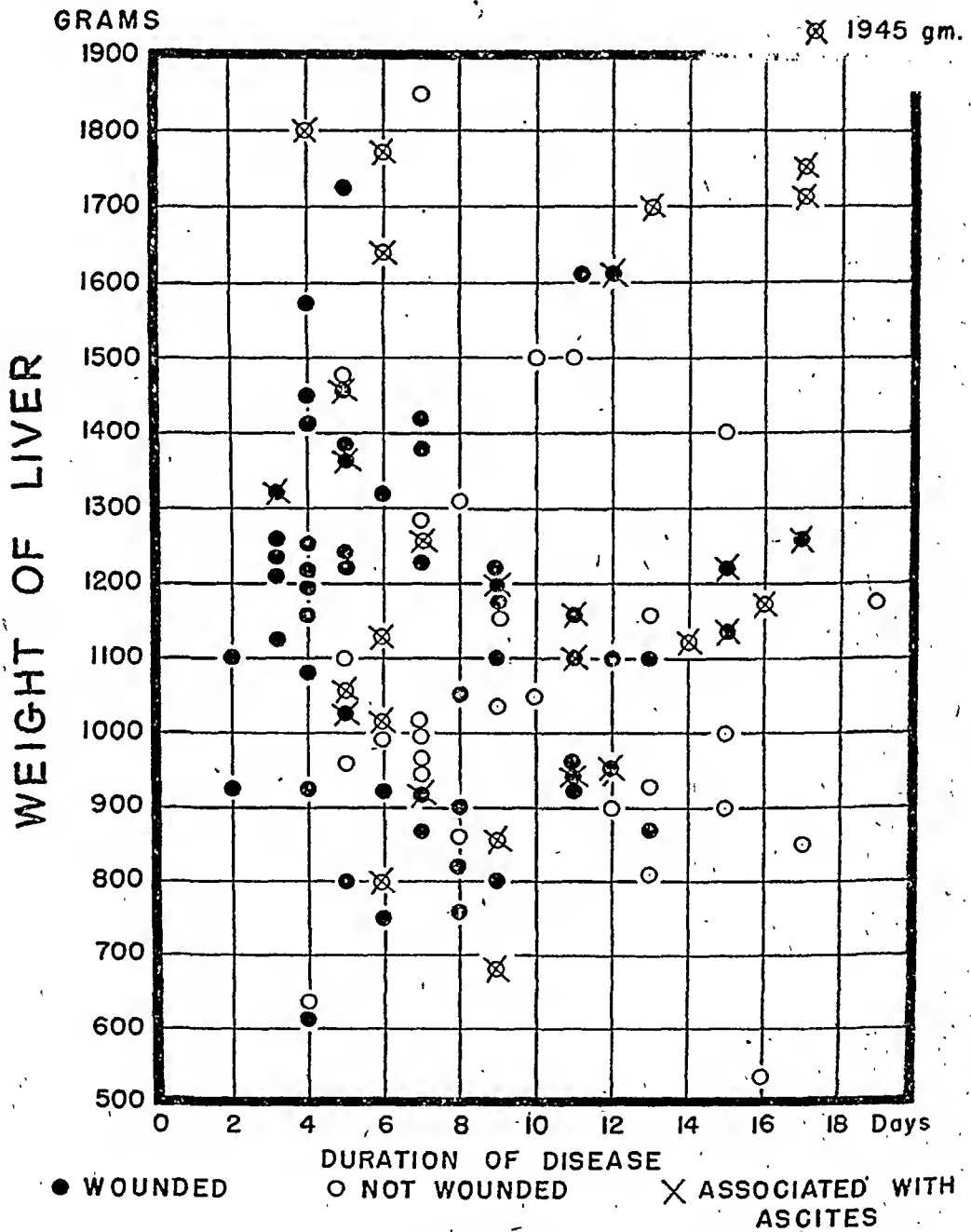
The differences in the incidence of ascites in the wounded and nonwounded groups are probably due to the more rapid course of the disease in the former.

### SPLEEN

In approximately two-thirds of the cases the spleen was enlarged and its weight exceeded 200 gm.; in about one-fourth it weighed more than 300 gm. (Table XIII).

Detailed information pertaining to the relation of weight to duration for the wounded and nonwounded groups is given in Text-Figure 8.

It is clear that in these fulminant cases there is no definite relation of weights either to duration of illness or to the epidemiologic forms of hepatitis. For example, in the 6 cases in which death occurred 3 days



Text-Fig. 7. Distribution of hepatic weights in respect to duration of disease for wounded and nonwounded patients.

after onset of symptoms, 3 of the spleens weighed approximately 450 gm., the 3 others less than one-half as much. The somewhat greater number of large spleens among the wounded probably is relative to the preponderance of this group.

Grossly, the spleens in the fulminant cases usually had a tense capsule, but when bisected were found to be soft. The cut surface was dark red and congested. The follicles were large and in many instances contained a central opaque fleck.

Microscopically, the majority presented a well marked hyperplasia of reticulo-endothelial and lymphoid components. The follicles usually were prominent and their germinal centers were enlarged and often degenerated or necrotic (Fig. 27). Similar small focal areas of necrosis were frequently observed in the pulp. Occasionally the organ was conspicuously infiltrated with eosinophils and plasma cells. Nearly all spleens were greatly congested, often to such an extent that small

TABLE XIII  
*Weight of Spleen in 94 Cases of Fulminant Hepatitis*

	Number of cases
(gm.)	
To 99	2
100—199	28
200—299	43
300—399	7
400—499	9
Over 500	5

hemorrhages had occurred. Rigidity of the sinuses with depletion of pulp, such as was the rule in the subacute cases, was not observed.

The common enlargement of the spleen in fulminant hepatitis is due to hyperplasia of its component cells and to acute congestion.

#### INTESTINES

Phlegmonous inflammation of the colon, which occurred in 15 per cent of the subacute cases,<sup>24</sup> was not present in any fulminant ones. Occasionally there was a moderate degree of edema, always associated with ascites. Mucosal hemorrhages were equally as extensive as in the earlier series.

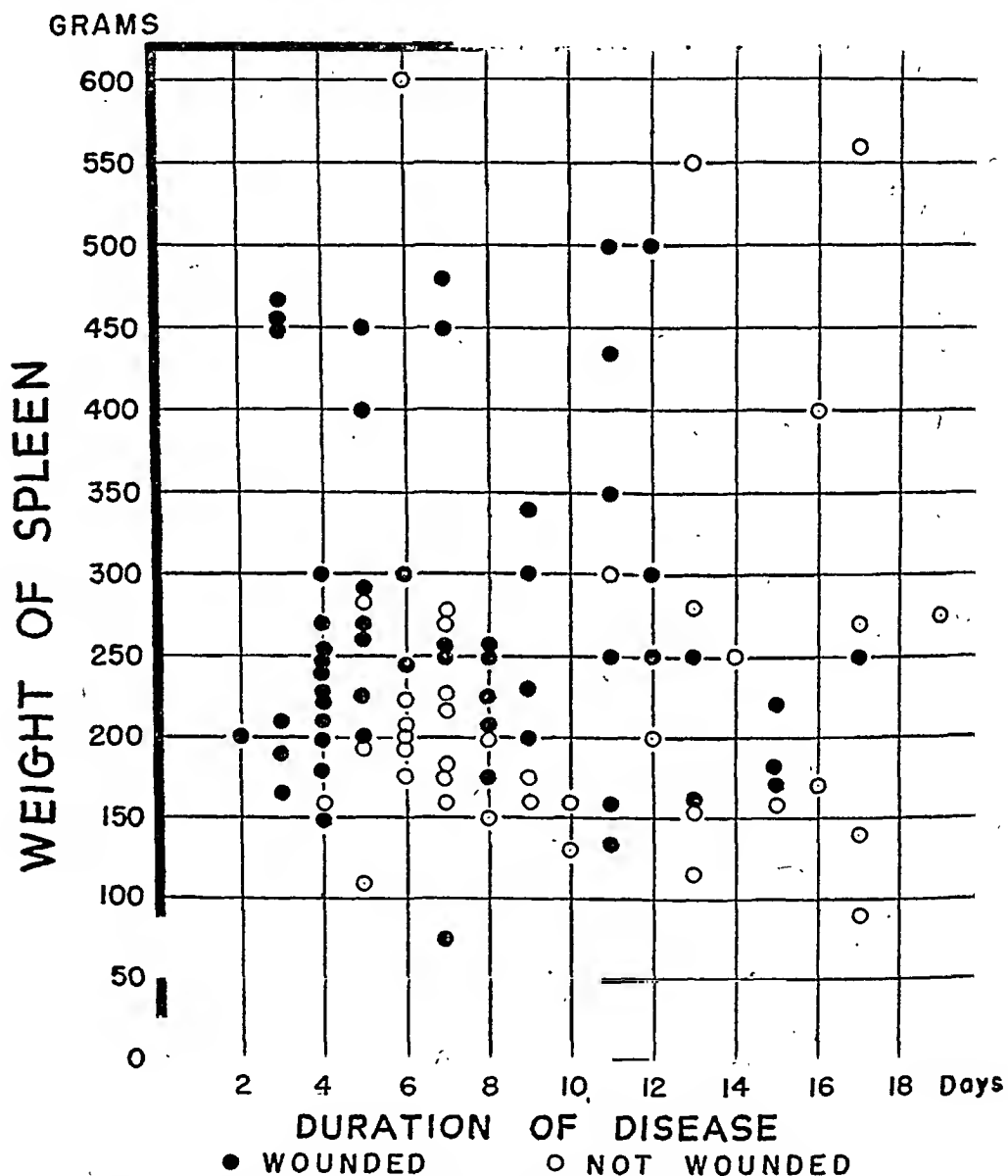
#### KIDNEYS

The kidneys were slightly swollen in most cases, 20 per cent being of normal size, 40 per cent weighing from 350 to 400 gm., and the remaining 40 per cent slightly above 400 gm.; no enlargement over 500 gm. was recorded. The variation in color was wide; some kidneys were dark from congestion; others were pale. In very few was any bile discoloration evident, and then it was confined to tinting of the pelvic epithelium; none was deeply bile-stained.

Microscopically, as observed in routine paraffin sections, there were few conspicuous changes. The glomeruli were uniformly normal except



for the presence in some instances of albuminous precipitates in the capsular spaces. The epithelium of the proximal convoluted tubules was generally slightly swollen and frequently showed basal vacuolization; the nuclei were well preserved and normally chromatic. Very



Text-Fig. 8. Distribution of splenic weights in respect to duration of disease for wounded and nonwounded patients.

little bile staining could be recognized in the epithelial cells, and bile-stained casts were seldom found. No severe degenerative changes and no necrosis were present at any level of the tubules. There was no interstitial reaction.

Fat deposits were usually conspicuous when frozen sections were stained with sudan stains. They were most marked in the proximal convoluted tubules (Figs. 28 and 29), beginning in the neck and con-

tinuing through the entire length; were less constant and less severe in the ascending limbs of Henle (Fig. 30), and still less so in the distal convoluted tubules. The deposits were always most apparent in the basal portion of the cells; the individual droplets were generally minute, but some attained the diameter of the nucleus. No fat was seen in the glomeruli, in the descending limbs of Henle, in the collecting tubules, or in the interstitial tissues. With polarized light the fat did not prove doubly refractile.

The degree of deposition of fat was estimated on a 1 to 3 plus scale in 39 cases chosen at random. No relation was found between its intensity and the degree of nitrogen retention, but when the amount of fat vacuolization was compared with duration of disease a distinct relationship became apparent. In 23 cases showing heavy deposits the average duration of hepatitis was 6 days. No fat, or only traces of it, was observed in 16 cases in which the average survival period was 11 days. Either the process is more severe when hepatitis is most fulminant, or it is a transitory phenomenon which tends to disappear with the passage of time. The lack of other evidences of epithelial degeneration suggests that it is a storage phenomenon, possibly dependent on the sudden liberation of large amounts of fat from the destroyed liver cells.

The absence or mildness of the degeneration observed in this series contrasts sharply with the severe grades of bile nephrosis noted in the subacute cases previously reported.<sup>24</sup>

#### BRAIN

The brain grossly presented no significant changes. Particularly noteworthy was the absence of hemorrhage. Microscopically, a non-specific encephalopathy of mild degree was nearly always observed. In the most fulminant cases the bodies of many ganglion cells were swollen, the nuclei distorted and their chromatin granules dispersed and scattered. When the disease lasted more than 10 days some ganglion cells usually were shrunken and had dense pyknotic nuclei. Satellites and neuronophagy were moderate in some locations, but nowhere were they conspicuous. No perivascular round cell infiltrations, such as in the subacute form, were observed either in the meninges or the nervous system proper.

The glia presented interesting alterations. "Naked nuclei" were frequently numerous; they were poorly chromatic, somewhat swollen, and often slightly distorted. Nuclei of this kind have been described in many forms of hepatic disease; they are, however, not specific. The glial reaction was most evident in the basal ganglia.

Despite the prominence of nervous manifestations in the terminal

stage of hepatitis, the histologic changes usually are not impressive. This has been attributed to the tempo of the disease, for cerebral manifestations as a rule precede death by but few days.<sup>41</sup>

### CLINICOPATHOLOGIC CORRELATIONS AND DISCUSSION

Fulminant hepatitis differs in numerous features from the subacute form which, until 2 or 3 years ago, had prevailed in all recorded epidemics. Is it a different disease? Are particular etiologic or epidemiologic factors responsible for its fulminant character? What is the mechanism of jaundice and of ascites in this form? What effect does rapid destruction of the liver have on the kidney? These are the questions we have selected for brief discussion. None can adequately be answered; rather, discussion can only indicate the need for further investigation.

#### *Clinical and Epidemiologic Forms of Epidemic Hepatitis*

Clinically, the fulminant form of hepatitis is characterized by the short and stormy course, the frequency of fever during the prodromal stage, and the brevity of the icteric stage. Biochemically, the fall in blood sugar and the low value of blood urea nitrogen reflect rapid and massive necrosis of the hepatic parenchyma. Pathologically, the outstanding lesions are extensive and comprise uniform destruction of liver cells, minimal evidence of regeneration, and marked inflammatory infiltration especially of the portal areas and the perilobular boundaries.

Consideration must be given the possibility that we are dealing with a disease different from epidemic hepatitis. Against this hypothesis two arguments can be raised. First, the similarities are greater than the differences, and these differences, in turn, are quantitative rather than qualitative. No new features, either clinical or pathological, have appeared in the current form, although some have been exaggerated or minimized. Second, all grades of transition between the fulminant and subacute forms have been observed. The conclusion is warranted, therefore, that fulminant hepatitis is a form of epidemic hepatitis, although final proof must wait upon advances in the determination of etiologic factors and upon controlled experimentation.

The specific causal agent of epidemic hepatitis has the attributes of a filtrable virus.<sup>28,37,42-45</sup> The virus has not as yet been cultivated, nor has any animal susceptible to it been found.<sup>5,43</sup> At present the only means of demonstrating the virus is by human transmission; in recent years this has been accomplished with volunteers.<sup>33,42,44,46</sup> The infectious agent has been shown to exist in the blood, the feces, the urine,

and nasal washings. Under natural conditions the disease probably is transmitted either through the alimentary tract by ingestion of contaminated food or water, or through the nasopharynx as droplet infection.<sup>5,28,44</sup> Under artificial conditions it may be transmitted by parenteral injection of blood, plasma, or serum from an individual harboring the virus.

Two main epidemiologic variants of the disease may be recognized: naturally occurring hepatitis (in endemic or epidemic form), and inoculation hepatitis. The latter may develop after transfusions of blood or its derivatives (homologous serum hepatitis),<sup>5,27,29,30,32,42,54</sup> after inoculation of vaccine containing small amounts of infectious serum (post-vaccinial hepatitis),<sup>5,11,47,48</sup> after injections of therapeutic agents through needles or syringes contaminated with blood from an infected person (hepatitis after insulin injection, post-arsphenamine hepatitis).<sup>49-51</sup> Transmission experiments indicate that the specific causal agents of these variants are similar or closely related.<sup>33,37,42,45,46</sup>

From the epidemiologic point of view the present series, regardless of duration, comprises 57 per cent of spontaneous (*i.e.*, naturally-occurring) cases (of which 16 per cent are regarded as endemic and 41 per cent as epidemic), and 43 per cent are believed to represent homologous serum hepatitis. Cases of the controversial post-arsphenamine hepatitis have been excluded, and there are no examples of post-vaccinial hepatitis. This study has shown that in the fulminant form the manifestations of the disease and the lesions of spontaneous and transfusion hepatitis are in every respect indistinguishable. Similarly, the less fulminant examples of this series present pathologic changes indistinguishable from those observed in cases of comparable duration in the 1942 epidemic (in which the majority could be regarded as post-vaccinial hepatitis), and from those reported in the Swedish epidemic of 1927 (which represented the naturally-occurring disease). These findings are in agreement with clinical observations<sup>5,29,31,32,50,51</sup> and with biopsy studies of nonfatal cases.<sup>19,20,52</sup>

It is concluded that the epidemiologic variants of the disease do not differ in their clinical manifestations.

#### *Factors Responsible for the Fulminant Form of Fatal Hepatitis*

Epidemic hepatitis is the only disease of this war which has become pandemic. With widespread prevalence there are indications that the disease has become more severe; thus many observers have reported that in a high proportion of cases, instead of the common insidious onset, the symptoms are those of an acute febrile infectious disease. Yet the mortality rate, so far as can be judged from figures at present

available, has not significantly altered. However, the course of fatal hepatitis has been more fulminant during the past 2 or 3 years. To what this may be attributed—whether to host factors, which have promoted an increase in individual susceptibility, or to changes in the infectious agent—is difficult to assess. It is not known whether a fulminant course is associated with an especially virulent strain or an especially large dose of virus. As to host factors which may increase susceptibility to the infection, either in fatal or nonfatal cases, a number have been suggested: age, physical strain, diet, other diseases,<sup>5</sup> combat fatigue,<sup>8,12</sup> accidental variation in resistance,<sup>2</sup> and a poor nutritional state.<sup>3</sup> In the present series we may at least rule out the factors of age, climate, or other variables depending on geographic location. All the other factors may well have played a part. They are interrelated; the most tangible ones are the effects of trauma and consequent transfusions, and the effects of nutritional disturbances. Even if we cannot, at this time, arrive at the correct evaluation of these factors, a discussion of them may point the way to future inquiry.

### *Trauma and Transfusion*

Ninety per cent of the cases in this series occurred among combat troops in overseas theaters of war. Of the fulminant group nearly two-thirds (61 per cent) had sustained severe battle injuries or extensive burns; in sharp contrast, only 2 per cent of the group which survived for more than 20 days had been injured. Practically all casualties may be assumed to have received transfusions of blood or its derivatives. It is thus possible that the specific agent of hepatitis was transmitted directly and perhaps in large amounts. But tens of thousands of other men have sustained equally severe wounds, and have received transfusions without developing hepatitis. There is no accurate information as to how often hepatitis did develop. We do know that during the period of this study, covering nearly 2 years, the disease was fatal in less than 100 individuals who had been wounded and transfused.

Trauma, directly, cannot be implicated as the cause of fulminant hepatitis, but it may be important because it lowers individual host resistance in some manner.

The evidence that an infectious agent was transmitted by means of transfusion is circumstantial. It rests upon the well documented fact that the interval between parenteral introduction of virus and outbreak of symptoms, for all types of inoculation hepatitis, usually falls within a period of from 2 to 4 months.<sup>27,29,37,42,47,48</sup> The incubation period for the naturally-occurring disease is much shorter, and on the average

runs from 20 to 40 days.<sup>37,42,46</sup> The intervals between trauma and onset of symptoms in the present series is shown for 64 cases of fulminant hepatitis in Table XIV. It is a safe assumption that, for the majority of the casualties, plasma and transfusion therapy was concentrated within a few days of injury. Six cases of burns have not been included in this tabulation since these patients received plasma and whole blood for many weeks. From inspection of Table XIV it is evident that in the majority (69 per cent) the interval between transfusion therapy and onset of the disease fell within the limits of 50 and 89 days, the median duration being 70 days. It is very prob-

TABLE XIV  
*Interval between Receiving Wound and Initial Symptoms in 64 Cases of Fulminant Hepatitis*

Interval (Days) -	Number of cases	Per cent of cases
30-39	4	6
40-49	4	6
50-59	11	17
60-69	11	17
70-79	10	16
80-89	12	19
90-99	6	9
100-109	4	6
Over-110	2	3

able, therefore, that in these cases we are dealing with examples of hepatitis in which the virus was transmitted parenterally and not by "natural" means.

One distinct difference between naturally-occurring hepatitis and serum hepatitis in this series is the duration of disease; the course of the serum type tends to be more rapid (see Tables II and IV and Text-Figs. 1 to 3). Whether this is a general characteristic of this type of hepatitis cannot yet be stated with certainty; it may depend on the particular strains of virus injected, and on nonspecific individual host factors incident to combat trauma. In this connection the classical experiments of Opie<sup>53</sup> may be recalled. He demonstrated that the activity of a hepatic poison may be so enhanced by bacterial infection that a quantity of the poison which alone produces little change may, in combination with infective agents, cause destruction of almost the entire hepatic parenchyma. Opie believed it probable that those instances of acute yellow atrophy which accompany streptococcic infections are dependent upon some disturbance of metabolism or other form of intoxication which renders the liver unusually susceptible. Pertinent, too, are experiments of Bennett, Drinker, and Warren<sup>55</sup> on the effects of certain chlorinated hydrocarbons which may produce

extensive necrosis of the liver. These compounds are extensively used in industry, but the incidence of "acute yellow atrophy" in workers exposed to the compounds is very low. This fact suggests that certain individuals may be more susceptible, or that in fatal cases liver damage may have been augmented by some other agent. The latter hypothesis was tested by experiments on rats with confirmatory results; the effect of the compounds under discussion was found to be much enhanced by small sublethal doses of other hepatic poisons.

### *Nutritional Disturbances*

It is well known that liver injury may be produced by purely nutritional factors.<sup>56,57</sup> Several observers have implicated improper or inadequate diet and poor nutritional states as increasing susceptibility to the infectious agent of hepatitis.<sup>3,5</sup> What actual part such factors have played in the present series cannot be determined. Even on an adequate Army diet, some soldiers lose appetite and weight; it may be assumed that the strain and the exigencies of warfare have brought about very considerable disturbances of the nutritional state, and thus, perhaps, of resistance to the infectious agent. The recent experiments of Glynn and Himsworth<sup>58,59</sup> have great interest in relation to this problem. These investigators demonstrated that massive necrosis may be produced in rats without the intervention of any exogenous toxic agent, chemical or infective, solely as the result of a deficient diet. The essential factor in which it is deficient appears to be an amino acid. The destruction of hepatic parenchyma in these rats has many resemblances to that of fatal epidemic hepatitis in man. It develops abruptly after a latent period of several weeks, during which the liver is morphologically normal. The latent period probably represents the time required to deplete the reserve of certain essential constituents in the body's store of labile proteins. The lesions appear in two forms: a generalized massive necrosis when the diet is grossly deficient in necessary protein constituents, or a necrosis confined to certain parts of the liver, especially the left lobe, when the diet is less deficient but still not adequate.

### *Mechanism of Jaundice*

In the previous study of hepatitis<sup>24</sup> the general mechanism of jaundice has been discussed. Here it remains to inquire whether the same mechanism is operating in the rapidly destructive fulminant form of the disease.

In the subacute form of hepatitis the cause of jaundice appears to be principally a mechanical obstruction by bile "thrombi" of the

intralobular canaliculi in the surviving or regenerating liver tissue. Similarly, in liver taken for biopsy from nonfatal cases the canaliculi are occluded by plugs of altered and inspissated bile.<sup>40</sup> In fulminant hepatitis destruction of liver cells is often complete; at best only small rims of peripheral cells are spared. Necrosis of the hepatic columns leads, of course, to destruction of the delicate bile clefts, the canaliculi; obstructive factors cannot, therefore, be considered. Jaundice can only be accounted for by the rapid destruction of liver cells; the absence of liver cells makes it impossible to remove bilirubin from the circulating blood (or from the Kupffer cells).

But what of fulminant hepatitis without jaundice? This explanation gives no clue to the situation there. Careful study of the several examples in this series disclosed no differences in lesions. In examples included among representative cases (nos. 2, 5, and 7), the duration of the disease was 3, 4, and 6 days respectively, a sufficient time for jaundice to have appeared. No explanation can be offered for the absence of jaundice despite complete destruction of liver. Even though anicteric fatal hepatitis is the exception, it indicates the need for re-investigation of the complex mechanism of jaundice.

### *Ascites*

The mechanism of ascites is equally complex. It is surprising to find that approximately one-fourth of the fulminant cases are associated with ascites. Its incidence is definitely correlated with duration of disease, rising to 70 per cent in subacute hepatitis. This suggests that the factors which lead to the accumulation of fluid become more pronounced as the disease is protracted. There is also a correlation between ascites and weight of liver, in that ascites is most frequently associated with livers that are but little shrunken. As has been shown, the weight of the liver in the fulminant group is largely a function of its blood content. Destruction of the columns of liver cells tends to bring about dilation and congestion of the sinusoids, that is to say, stasis. But stasis does not invariably occur, nor is it always uniformly distributed throughout the liver. It is possible that such variations in blood content of the liver are due to alterations in the action of the diaphragm. This muscle renders an important aid to venous flow; it has been likened to a hand which rhythmically squeezes a sponge filled with blood.<sup>60</sup> Since clinical evidence shows that ascites develops usually during the terminal phase of the disease, more or less simultaneously with disturbances of the central nervous system, there exists the possibility that diaphragmatic innervation and action may be impaired. Whatever the rôle of the diaphragm may be, it seems plausible



that interference with venous escape from the liver is one of the chief causes of the ascites of fulminant hepatitis.

One other point deserves mention. In the experimental massive necrosis resulting from dietary deficiency, ascites commonly develops.<sup>58</sup> Mann and Bollman also have pointed out that ascites may be produced by dietary means.<sup>56,57</sup> Whether and to what extent such factors operate in fulminant hepatitis is problematical. Disturbances of plasma proteins resulting from destruction of liver, and their bearing on ascites cannot, in our series, be properly evaluated.

### *Fulminant Hepatitis and the Hepatorenal Syndrome*

Does the massive and rapid disintegration of liver cells which is characteristic of fulminant hepatitis lead to the symptom complex known as the "hepatorenal syndrome"?

Heyd<sup>61</sup> is generally given credit for having focused attention, in 1924, on certain types of "liver death" most commonly seen following surgical operations upon the biliary tract or severe trauma to the liver. His original description was amplified in subsequent reports<sup>62</sup> and his concept has been added to and modified by numerous other authors, notably Cave,<sup>63</sup> Helwig and associates,<sup>64-66</sup> Boyce and McFetridge,<sup>67</sup> and Wilensky.<sup>68,69</sup> Most recent authors have emphasized a renal as well as a hepatic element, and the term "hepatorenal syndrome" has become widely used, but has never been clearly defined. The present consensus, as summarized by Thorn,<sup>70</sup> distinguishes two symptom complexes: one characterized by sudden onset of hyperthermia, delirium and coma; the second by renal failure and uremic death, often preceded by an episode of circulatory collapse or other shock-like state. Evidence for a renal component in the first syndrome is minimal and consists of vaguely described, nonspecific changes of kidney structure, falling within the limits of "cloudy swelling," or of bile nephrosis in cases in which jaundice precedes the acute episode. In the second syndrome, degenerative changes of greater severity have usually, though not invariably, been recorded, but no constant picture emerges from the reports. At the time when they were published, neither clinicians nor pathologists were aware of the profound renal damage which may follow shock, regardless of whether the liver is diseased or traumatized.

A sharp distinction must be made between the hepatorenal syndrome and bile, or cholemic, nephrosis. The former is ill defined, the latter well recognized.<sup>71-73</sup> Briefly stated, cholemic nephrosis is associated with prolonged jaundice; it occurred in most cases of subacute

hepatitis.<sup>74</sup> The essential lesions are tubular degeneration of varying severity, the presence of bile casts, and alterations in glomerular permeability. It is usually assumed, and experimental evidence supports the hypothesis,<sup>74</sup> that bile salts are injurious to renal epithelium and are the primary cause of degenerative damage. Since bile salts are formed in the liver, massive destruction of this organ would tend to lessen rather than to increase their excretion by the kidney. Clinical studies of renal function in jaundiced patients<sup>75,76</sup> likewise demonstrated renal damage and proved, furthermore, that it was ordinarily reversible since the albuminuria, cylindruria, and slight grades of azotemia cleared with disappearance of icterus. Nearly all authorities agree that simple bile nephrosis does not explain the severe renal insufficiency of the "hepatorenal syndrome."<sup>65,74</sup>

Two hypotheses have been repeatedly suggested to account for this syndrome: (a) that a toxic substance is elaborated by the sudden destruction of liver parenchyma, and (b) that a malfunctioning liver fails to detoxify an unknown nephrotoxic agent from some other source. If either of these hypotheses were correct, fulminant hepatitis with its massive and rapid destruction of liver parenchyma should provide many examples of the hepatorenal syndrome.

If one accepts a broad definition of this syndrome<sup>68,69</sup> and includes hyperthermic deaths and all forms and degrees of simultaneous or successive impairment of liver and kidney function, our data provide positive evidence for its existence in fulminant hepatitis since the majority of cases showed both clinical and histologic evidence of some renal injury. A definition so inclusive is, however, all but meaningless. If the more usual and narrower definition of the hepatorenal syndrome is accepted, namely, that of renal damage consequent to liver disease and so severe that it leads to uremic death, our data are essentially negative.

Pathologically, in the fulminant cases of hepatitis no significant renal changes other than storage of fat were observed and the decreasing degree of this phenomenon with longer periods of survival indicates that it was reversible and transitory in character. From the clinical point of view the only important evidence suggestive of renal insufficiency was azotemia, but in only 4 cases were nonprotein nitrogen levels above 60 mg. per cent recorded, with a maximal figure of 103 in one case. Azotemia of even higher levels would constitute inadequate proof of renal impairment in view of the rapid and extensive liberation of protein which must result from the massive necrosis of liver cells. There was no correlation between degree of fat storage

and nitrogen retention. It is concluded that fulminant hepatitis is not associated with the "hepatorenal syndrome" as this syndrome is usually defined.

### SUMMARY

A fulminant form of epidemic hepatitis which runs a fatal course in less than 10 days has appeared during the past 3 years. In a new series of 196 cases of fatal hepatitis which occurred in the U. S. Army between August, 1943, and April, 1945, approximately one-half (53 per cent) were of this type. By contrast, not a single such case was observed during the Army epidemic of 1942 and only one during the Swedish epidemic of 1927; then the median duration of fatal hepatitis exceeded 5 weeks. The clinical features and pathologic changes of the fulminant form differ significantly from those of the subacute variety which predominated in previous epidemics.

On the basis of epidemiology the present series includes 29 examples of the endemic and 72 of the epidemic variant of "spontaneous" hepatitis, and 77 cases presumed to be "homologous serum hepatitis" following trauma and transfusions of blood or blood derivatives. Analysis makes it evident that the epidemiologic type does not determine the clinical form of hepatitis, whether fulminant or more protracted.

This study is based principally on 94 cases in which the clinical course of the disease did not exceed 9 days. Thirty-nine others with a duration of from 10 to 19 days have been used to supply additional information, for many of them had lesions indistinguishable from those of the more fulminant form. The remainder of the series, *i.e.*, the subacute cases, which clinically and pathologically resemble those of the 1942 epidemic, are considered only in connection with certain analyses, such as the significance of geographic factors.

No precise information is available as to whether the mortality rates are the same or different in the several epidemiologic variants. The average mortality during the period covered by this study was 0.3 per cent. Serum hepatitis tended to run a considerably more rapid course than the naturally occurring disease, but otherwise there were no discernible differences, either clinical or pathologic, between these variants.

Clinically, fulminant hepatitis was characterized by a sharp and stormy course. It usually was ushered in by one of two syndromes: (1) an "infectious" type in which high fever, chilliness, malaise, and general aching dominated the picture, and (2) a "gastrointestinal" type with anorexia, nausea and epigastric discomfort in the foreground. These two types were represented in approximately equal proportions, and during various epidemics often occurred side by side. The subse-

quent clinical manifestations bore no relation to the prodromal symptoms. Because of the brevity of the course, the initial symptoms sometimes merged with those of the terminal stage.

Temperature records were available for 68 of the fulminant cases. In all but one the onset was febrile. The temperature ranged from  $95^{\circ}$  to  $104^{\circ}$  and averaged  $102^{\circ}$  F., fever declining as a rule with the onset of jaundice. During the final stage of the disease there was almost invariably a sharp rise in temperature coincident with profound cerebral disturbances.

In contrast to the deep jaundice commonly observed in the subacute form, the degree of jaundice in fulminant hepatitis was often mild. Several anicteric cases are included in this series.

Among noteworthy laboratory findings were moderate degrees of nitrogen retention and lowering of blood sugar.

Pathologically, lesions other than those in the liver were relatively slight; the changes found in "spontaneous" and in "inoculation" hepatitis were in every respect similar. The lesion of the liver was characterized by extreme and often complete destruction of hepatic cells, and by a marked inflammatory reaction. Typically, the involvement was uniform. The gross appearance of the liver was not pathognomonic and gave no indication either of the extent of parenchymatous destruction or of the degree of inflammatory infiltration. The organ usually was flaccid and moderately shrunken, and the capsule was smooth or finely wrinkled. The cut surface most often presented an exaggerated "nutmeg" pattern, though sometimes it resembled that of an acutely congested spleen.

Microscopically, the destructive process was limited specifically to liver cells. Even in the more rapidly fatal cases the earliest stages of cell disintegration could not be observed; the dead cells had undergone lysis and the resultant debris had already been removed. The inflammatory infiltration was most conspicuous at the lobular peripheries and less so within the lobular remnants. The predominating cells were mononuclear forms—reticulo-endothelial derivatives, plasma cells, and lymphocytes. Regenerative hyperplasia of surviving parenchyma was minimal and confined to biliary rather than to hepatic epithelium. There was often a marked disparity between the apparent age of the lesions and the duration of symptoms. The pathologic changes in the liver were, in many instances, obviously older than the clinical history suggested; less frequently the reverse was true.

The spleen usually showed acute congestion and hyperplasia of its component cells. Focal areas of necrosis were common in the follicles and in the pulp.

The kidneys in the majority of cases were the site of marked fat storage, especially within the cells of the proximal convoluted tubules. The storage was not associated with significant degenerative changes; it probably was the result of sudden liberation of large amounts of fat from the destroyed liver cells. There was no correlation between degree of fat storage and nitrogen retention. The rapid destruction of the hepatic parenchyma did not lead to the development of the "hepatorenal syndrome" as it is usually defined.

Despite the marked nervous disturbances in the terminal stage of hepatitis, histologic changes in the brain were usually slight and consisted of a mild nonspecific encephalopathy.

The mechanism of jaundice in fulminant hepatitis is complex. The extensive and often complete destruction of liver cells must be considered a chief cause. No adequate explanation can be offered for the occasional occurrence of entirely anicteric cases of fulminant hepatitis.

Ascites was present in approximately one-fourth of the cases of fulminant hepatitis. The principal factor in its production is believed to be acute venous stasis in the liver.

The factors responsible for the appearance, during recent epidemics, of hepatitis in a fulminant form are difficult to assess. It is suggested that more or less interrelated host factors, such as fatigue, trauma and nutritional disturbances, rather than the strain or the amount of the infectious agent, play a dominant part.

We wish to express our grateful appreciation to the Staff of the Army Medical Library for the translation of a Russian article and for bibliographic assistance.

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[ Illustrations follow ]



## DESCRIPTIONS OF PLATES

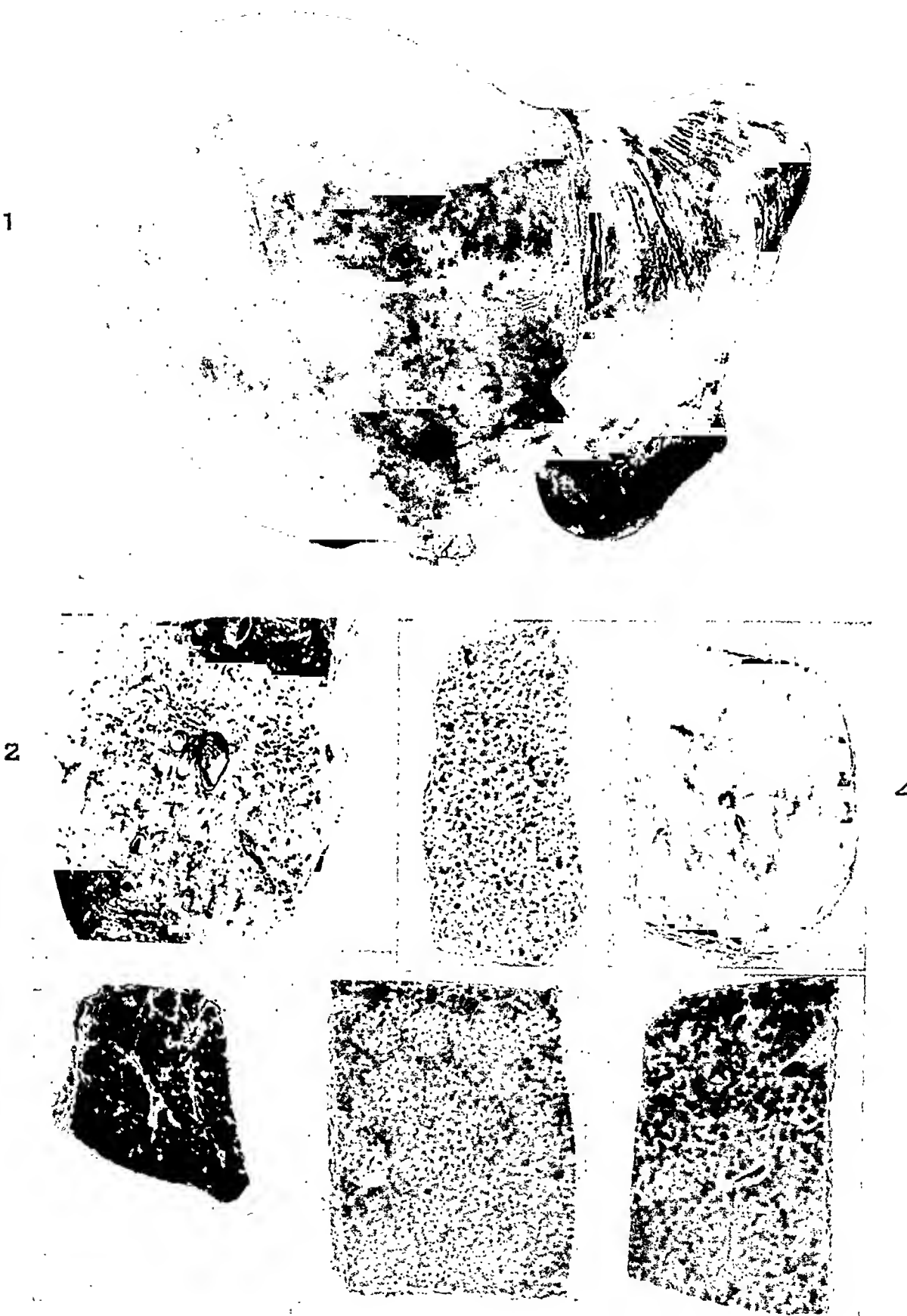
### PLATE 164

FIG. 1. Clinical duration of hepatitis, 4 days. Upper surface of the liver, which weighed 1200 gm. The surface of the right lobe is smooth; there are a number of subcapsular hemorrhages. The surface of the left lobe is finely wrinkled. (Army Institute of Pathology accession no. 148911.)

FIG. 2. Cut surface of liver shown in Figure 1. The appearance is similar to that of an acutely congested and hyperplastic spleen.

FIGS. 3 to 7. Representative areas of cut surfaces of livers from five cases of fulminant hepatitis; all have an exaggerated "nutmeg" mottling. Naked-eye examinations of these livers gave no indication of the extent of the parenchymatous destruction or of the prominence of inflammatory infiltration.

Fig.	Duration of disease	Weight of liver	Case no.
3	3 days	1205 gm.	126048
4	4 days	1200 gm.	126423
5	5 days	1025 gm.	128680
6	8 days	Normal size	103350
7	8 days	Shrunk	128577



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PLATE 165

FIG. 8. Duration of disease, clinically less than 1 day. (See case report 21.) Microscopic appearance of the liver at low magnification. The hepatic parenchyma has been destroyed. The portal regions and the perilobular boundaries are densely infiltrated with inflammatory cells. (See Fig. 15 for appearance of a lobule at higher magnification, Fig. 24 for details of the inflammatory reaction, and Fig. 27 for changes in spleen.)  $\times 25$ . (A.I.P. acc. 123465.)

FIG. 9. Duration of disease, 3 days. (See case report 1.) Photomicrograph of liver at low magnification. The hepatic cells have been destroyed. The lobular remnants are engorged with blood. The peripheries are outlined by bands of inflammatory cells. (Fig. 10 shows a portal region at higher magnification, Fig. 12 details of the cells, and Fig. 19 illustrates the observation that in areas of parenchymatous destruction the reticulum framework is preserved.)  $\times 25$ . (A.I.P. acc. 124057.)

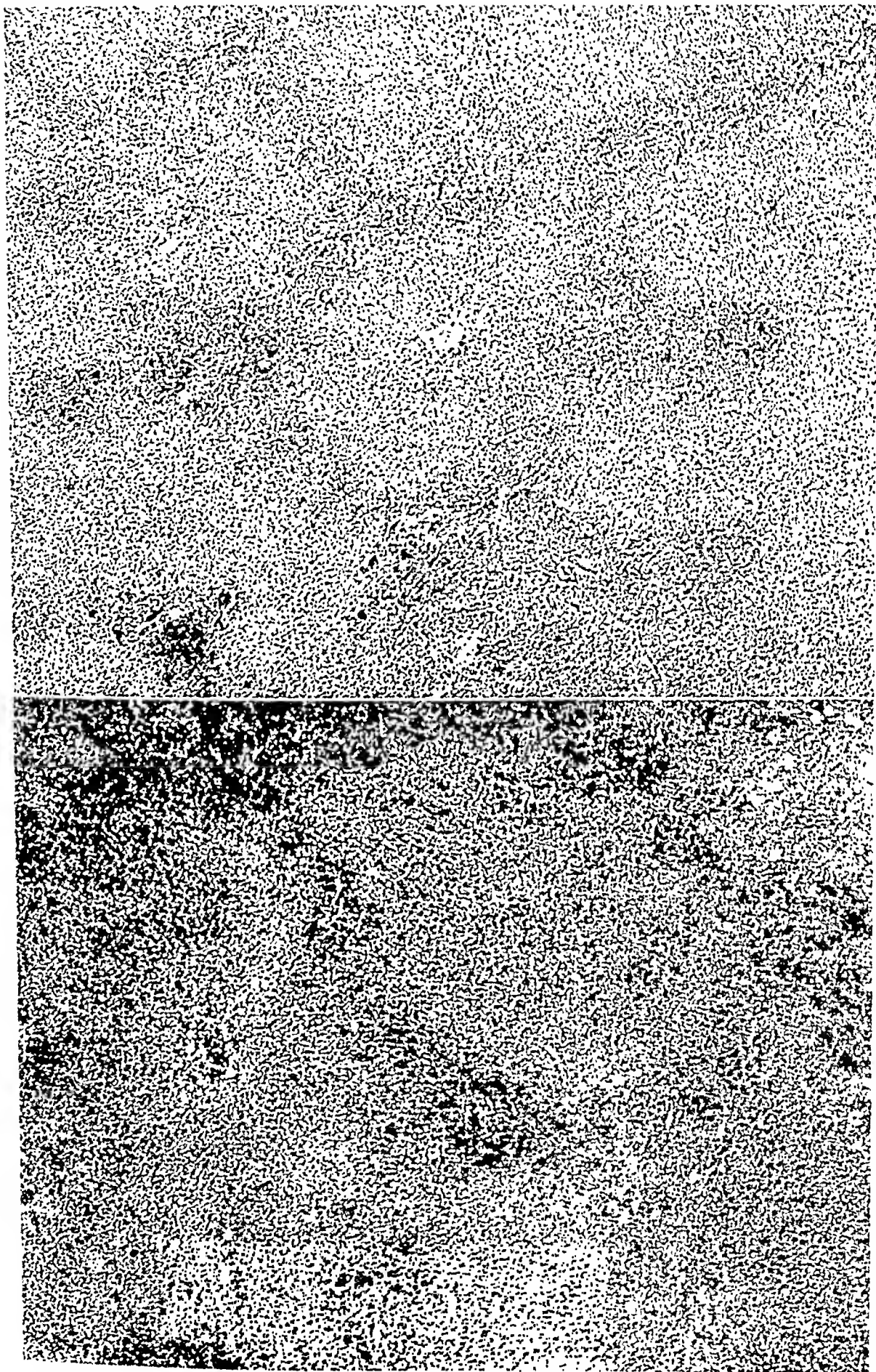
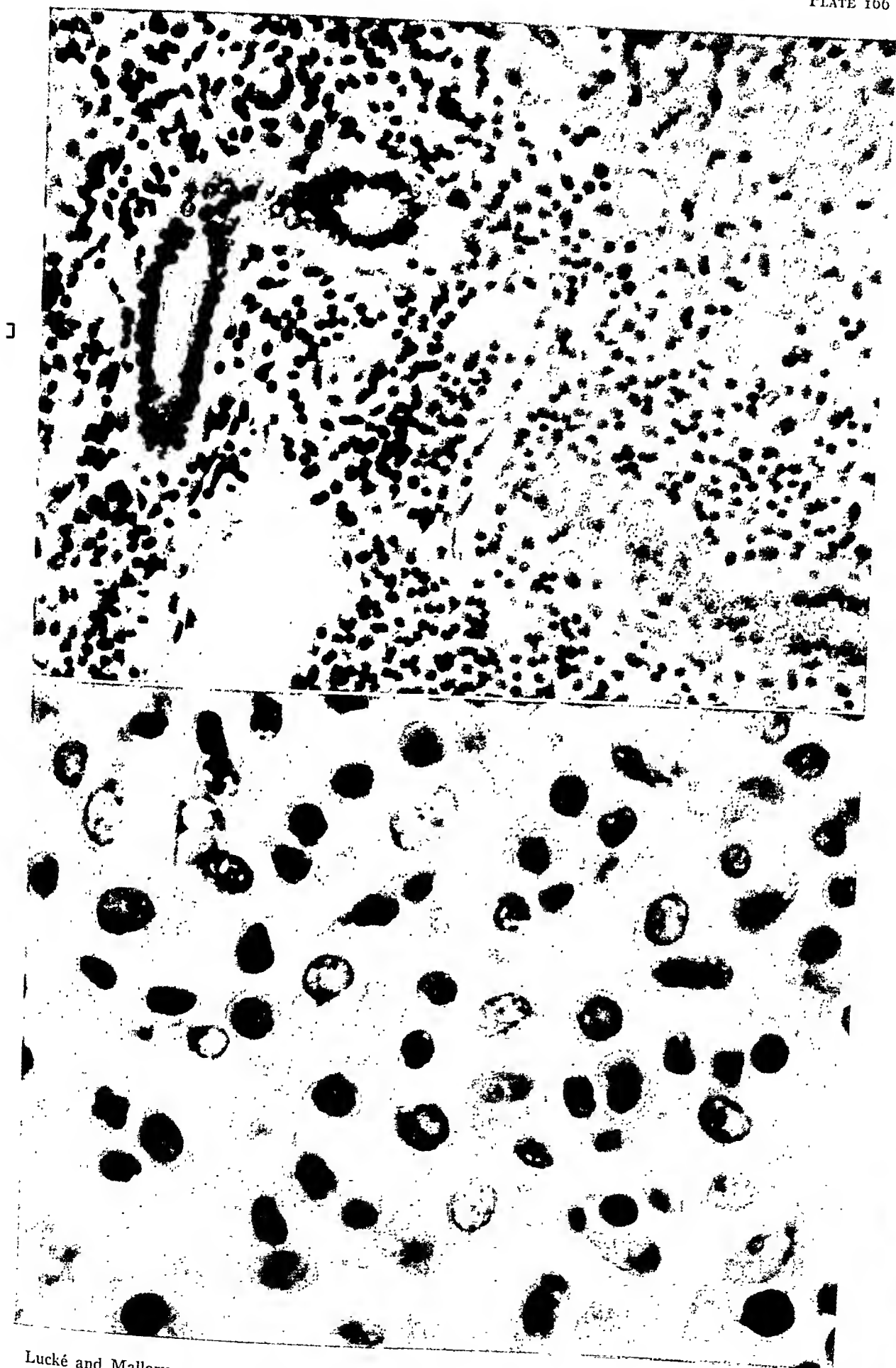


PLATE 166

FIG. 10. Duration of disease, 3 days. (See case 1.) Cell infiltration of the portal stroma at higher magnification than in the preceding photomicrograph. In the upper right-hand corner of the figure is shown the complete destruction of hepatic cells and great engorgement of the lobular remnants. Several bile ducts are sending out solid buds of proliferative epithelium. (See also Figs. 9, 12, and 19).  $\times 400$ . (A.I.P. acc. 124057.)

FIG. 11. Duration of disease, 9 days. (See case 13.) A representative picture of the cellular composition of the infiltrate at the lobular peripheries. Most of the cells are large mononuclears, but some plasma cells and lymphocytes are also present.  $\times 900$ . (A.I.P. acc. 114947.)

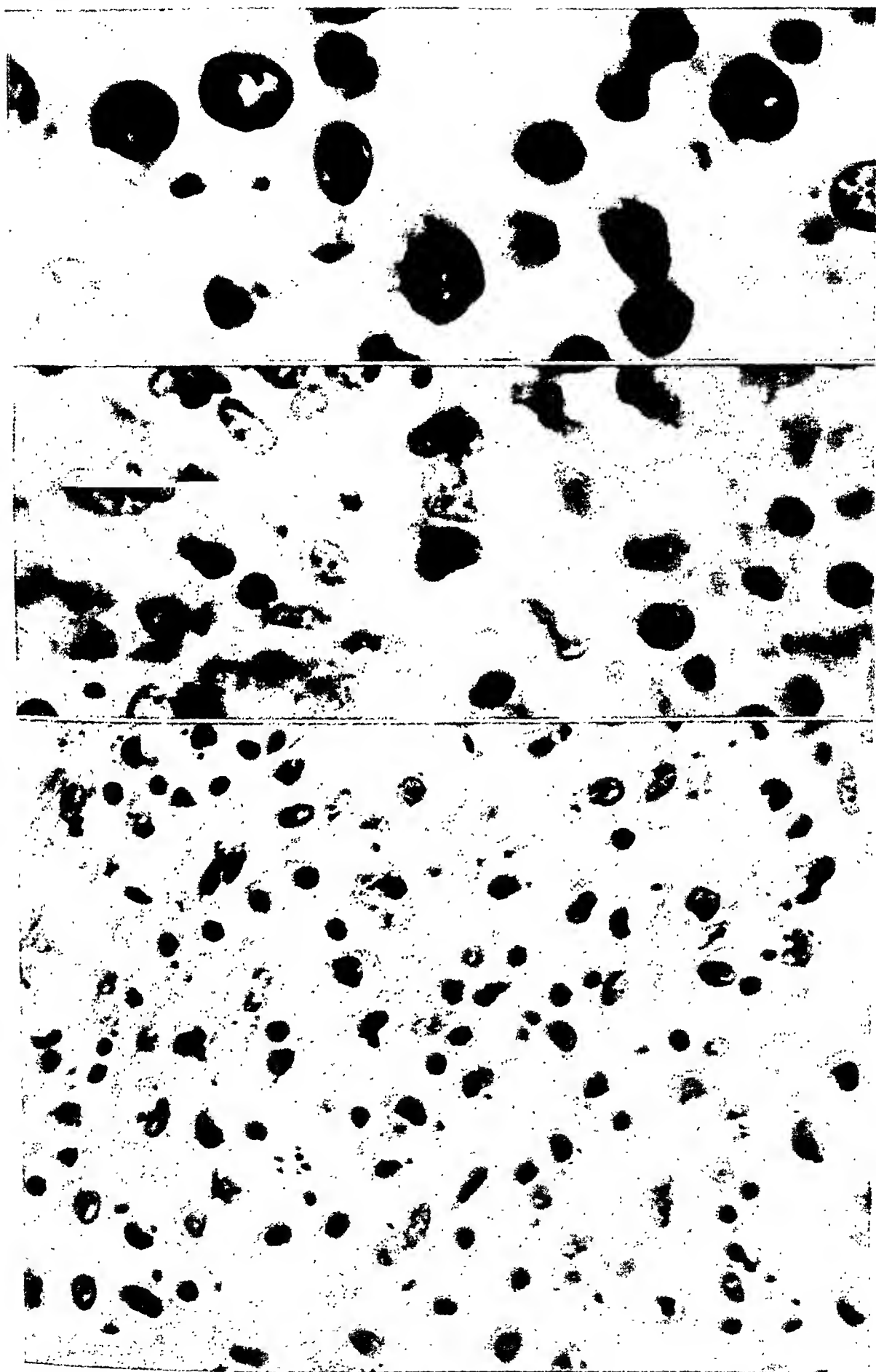


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PLATE 167

- FIG. 12. Duration of disease, 3 days. (See case 1.) The figure shows a group of plasma cells. In many cases these cells are numerically conspicuous, although they rarely predominate. (See also Figs. 9, 10, and 19.)  $\times 2280$ . (A.I.P. acc. 124057.)
- FIG. 13. Duration of disease, 7 days. Several eosinophils may be seen among the cells at a lobular periphery.  $\times 1300$ . (A.I.P. acc. 133951.)
- FIG. 14. Duration of disease, 9 days. Cell reaction within the lobular remnants. The predominant cells are mobilized and proliferated macrophages; many have ingested a brownish pigment, lipofuscin.  $\times 1300$ . (A.I.P. acc. 131876.)



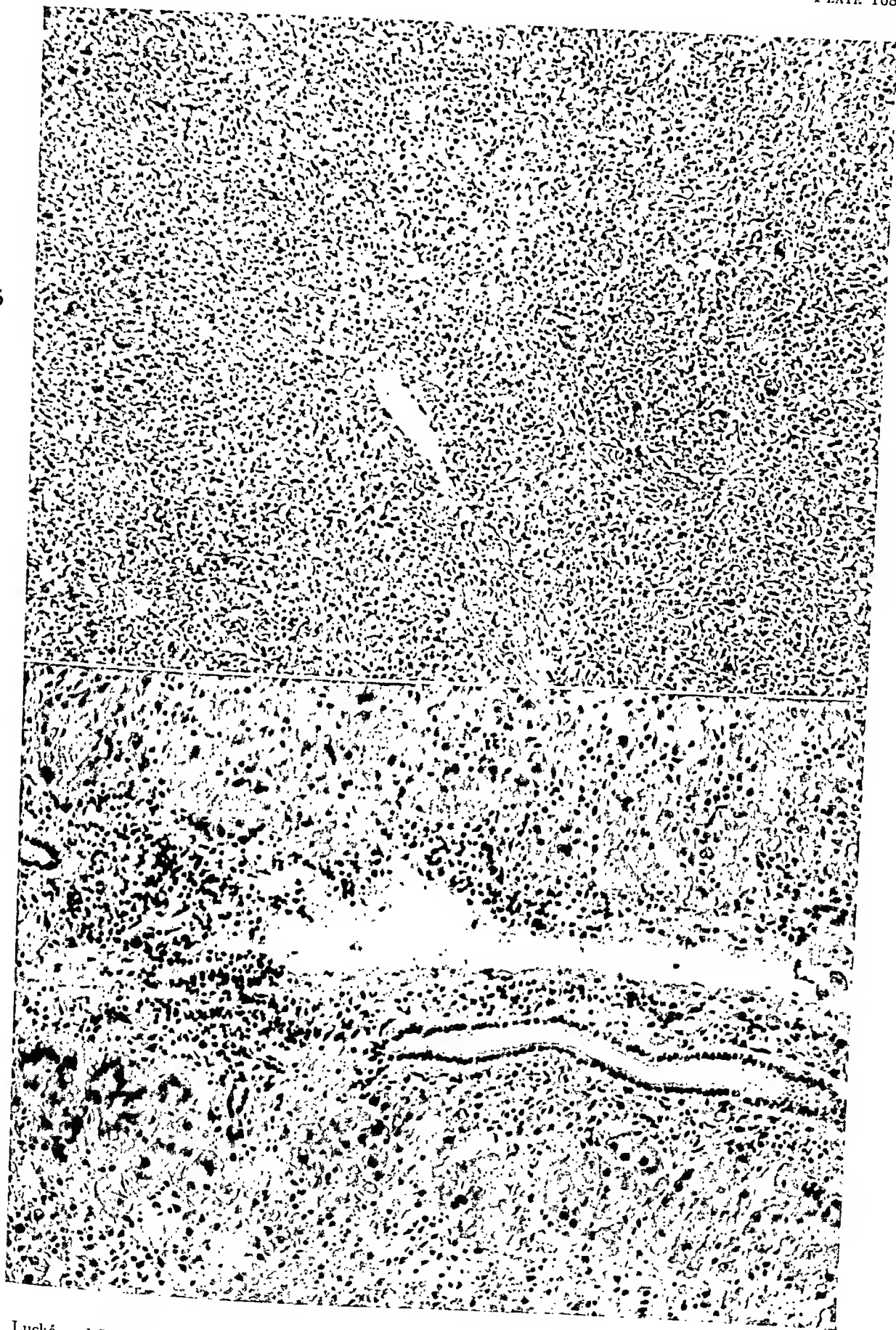


## PLATE 168

- FIG. 15. Duration of disease, clinically less than 1 day. (See case report 21.) A hepatic lobule at higher magnification than shown in Figure 8. In the photomicrograph the central lobular vein may easily be recognized; the lobular boundaries are indicated by small proliferating bile ducts, the significance of which is discussed in the text. The hepatic parenchyma has been destroyed; the lobular remnants are invaded by inflammatory cells, the nuclei of which appear as black dots. (See also Figs. 8, 24, and 27.)  $\times 145$ . (A.I.P. acc. 123465; neg. 86343.)
- FIG. 16. Duration of disease, 4 days. (See case report 17.) The figure shows a portal region with vein and bile duct cut longitudinally. The stroma is infiltrated with mononuclear cells which extend into the interior of the adjacent lobules. Isolated liver cells remain at the extreme periphery of the lobule; the great bulk of the parenchyma is destroyed.  $\times 175$ . (A.I.P. acc. 129051; neg. 86333.)

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Lucké and Mallory

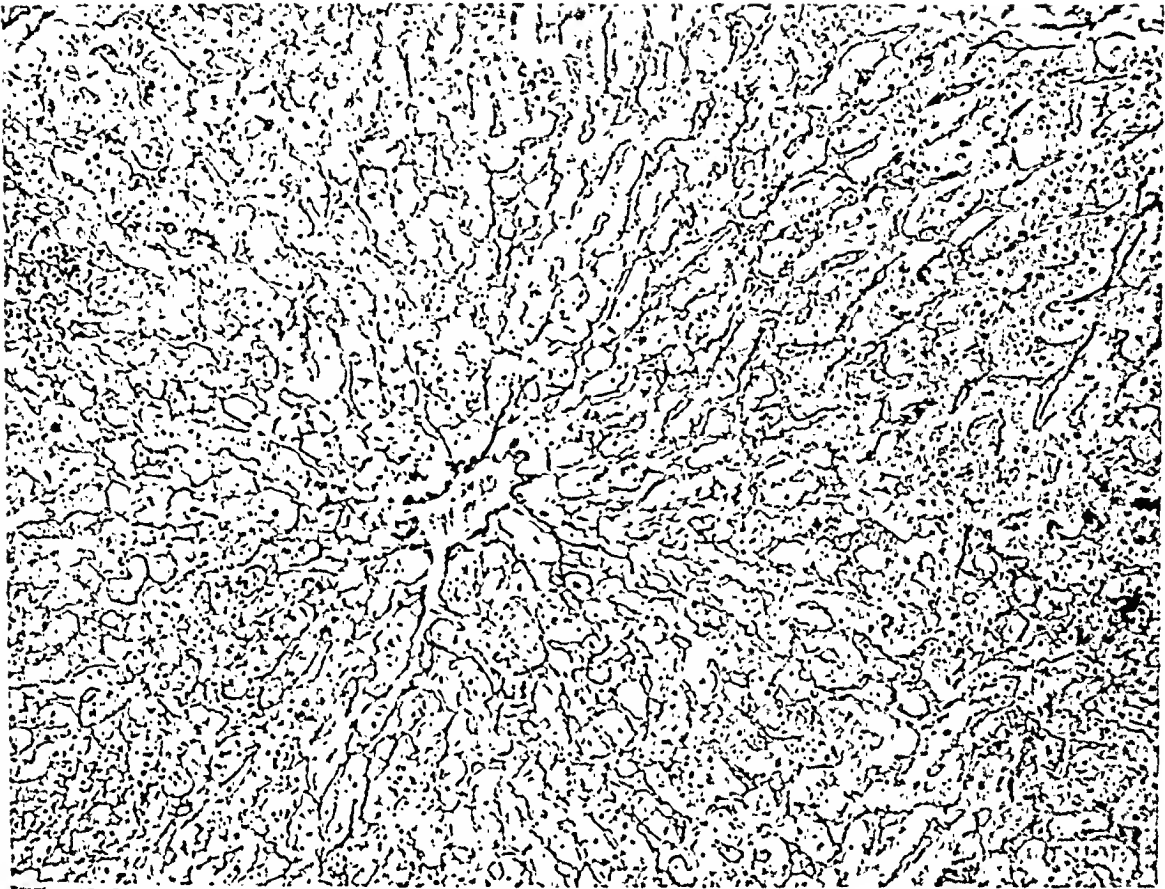
The Fulminant Form of Epidemic Hepatitis

PLATE 169

FIG. 17. Duration of disease, 3 days. (See case report 2.) The figure shows the intact reticulum frame of a lobule, the parenchyma of which has been completely destroyed. (See Fig. 3 for gross appearance of cut surface of liver; Fig. 18 for change in central lobular vein, and Fig. 23 for proliferation of septal bile ducts.) Wilder's reticulum stain.  $\times 145$ . (A.I.P. acc. 126048; neg. 86345.)

FIG. 18. Duration of disease, 3 days. (See case report 2.) A central lobular vein has a conspicuously thickened wall which appears hyalinized, and is invaded by a few inflammatory cells. The surrounding tissue is congested and infiltrated with mononuclear cells. The hepatic parenchyma has been destroyed. (See Fig. 17 which shows that the reticulum framework is preserved.)  $\times 205$ . (A.I.P. acc. 126048; neg. 86344.)

7



18

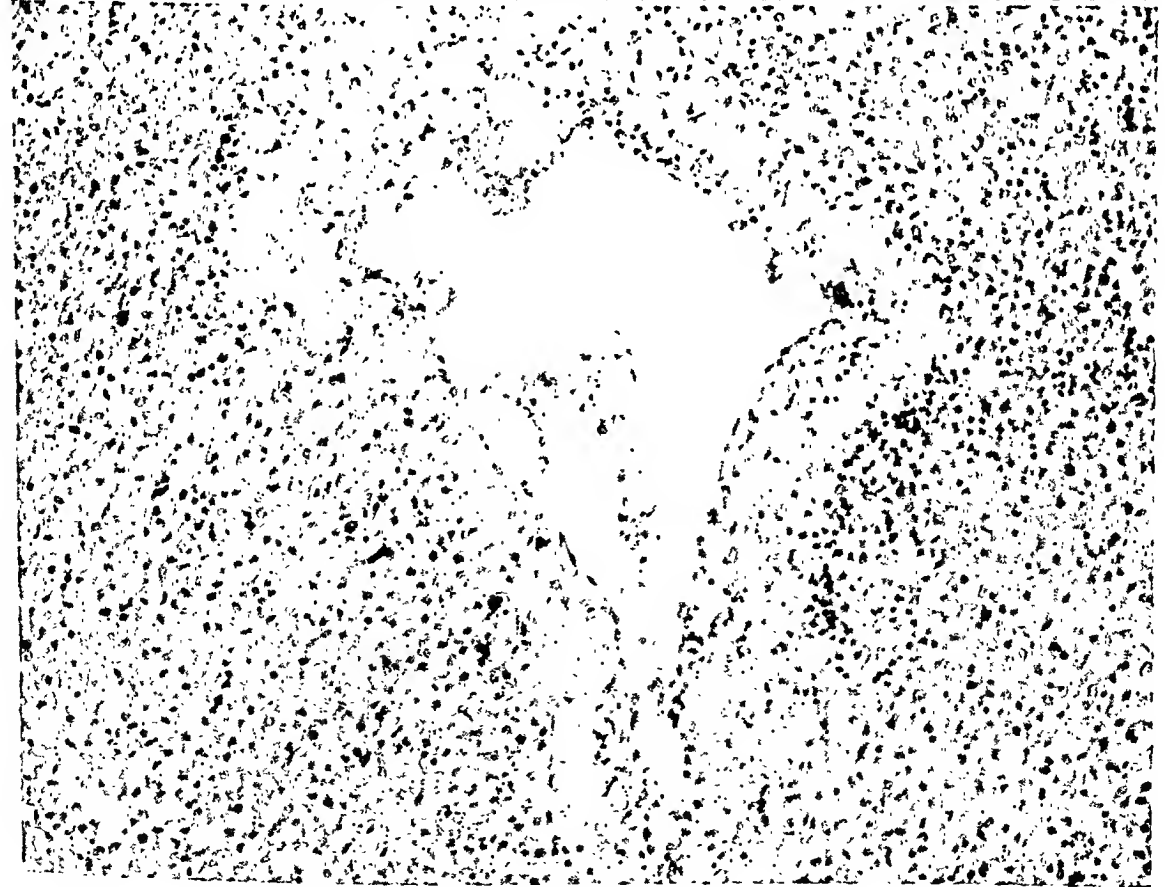


PLATE 170

FIG. 19. Duration of disease, 3 days. (See case report 1.) A hepatic lobule, the periphery of which is densely infiltrated with inflammatory cells which extend into the interior of the lobule. The hepatic parenchyma has been entirely destroyed and traces of liver cells have disappeared. The reticulum framework, however, was preserved. The lobular remnants are greatly engorged with blood. (See Fig. 9 for a photomicrograph in color showing a larger field at lower magnification, and Figs. 10 and 12 for cellular details.)  $\times 160$ . (A.I.P. acc. 124057; neg. 86346.)

FIG. 20. Duration of disease, 8 days. A portal triad at the junction of three adjacent lobules. There is a conspicuous inflammatory reaction which extends along the twigs of the bile ducts and vessels into the perilobular boundaries. The liver cells have disappeared. (See Fig. 7 for gross appearance of cut surface of liver.)  $\times 145$ . (A.I.P. acc. 128577; neg. 86336.)



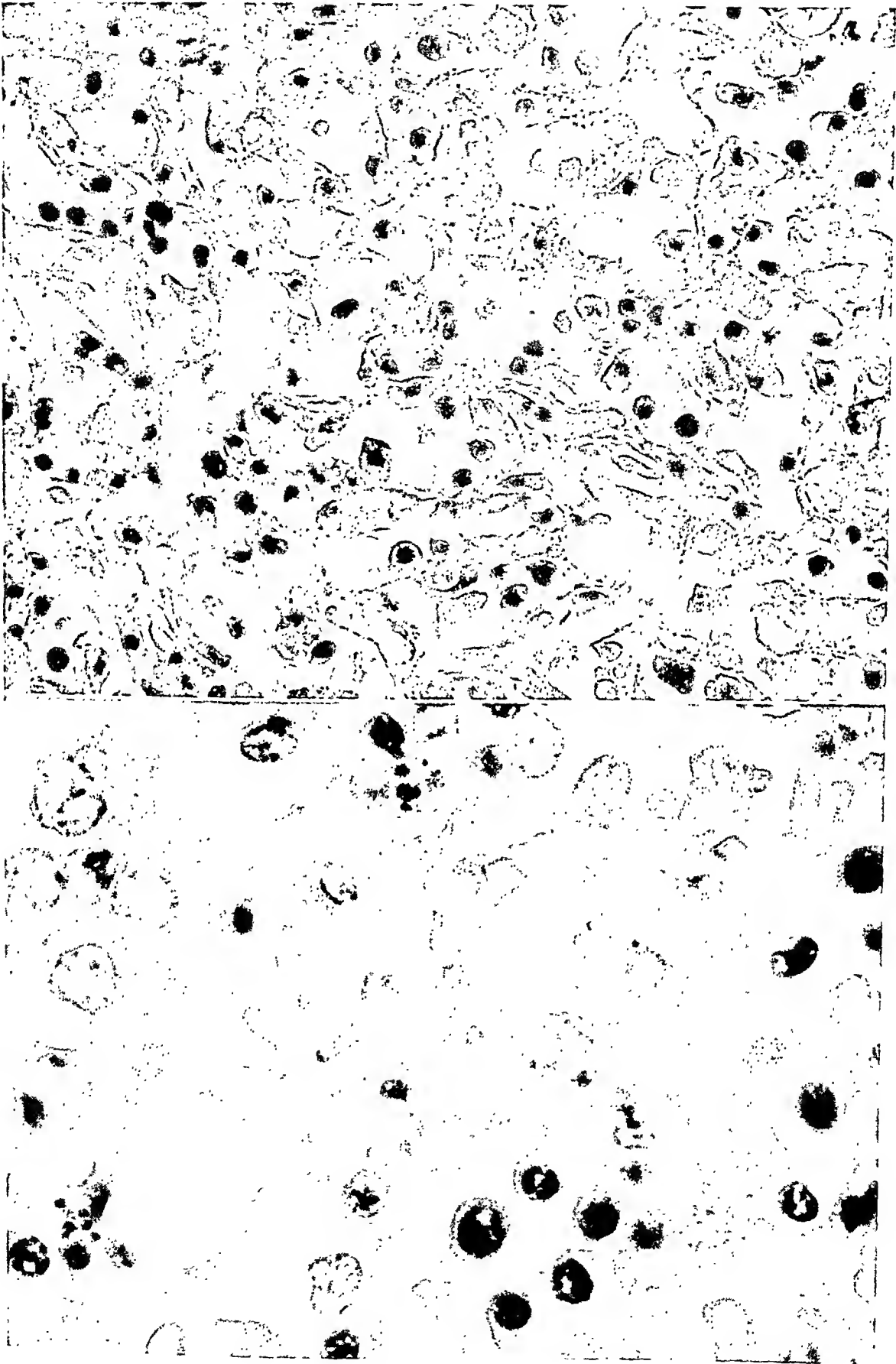
Lucké and Mallory

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PLATE 171

FIG. 21. Duration of disease, 7 days. Inflammatory reaction within a lobule. The parenchymatous cells have been destroyed and their débris has been removed. The section has been stained by Masson's trichrome method to bring out the boundaries of the sinusoids, and thus to show the distribution of the inflammatory cells. It will be noted, first, that the sinusoids are widely dilated (due to the loss of the hepatic columns); second, that the inflammatory cells (mostly mononuclear) lie both within and between the sinusoids; and third, that because of these changes, the hepatic tissue resembles splenic pulp. The dark appearance of some histiocytes is due to ingestion of a pigment, lipofuscin.  $\times 600$ . (A.I.P. acc. 126143; neg. 86326.)

FIG. 22. Duration of disease, 7 days. The figure shows a field near the periphery of a lobule. The parenchyma has been destroyed; the vascular stroma is invaded by plasma cells. The large coherent cells in the upper left of the photograph probably are proliferating biliary epithelium. In the upper center may be seen a congested sinusoid with intact walls.  $\times 1360$ . (A.I.P. acc. 133195; neg. 86342.)



2



PLATE 172

FIG. 23. Duration of disease, 3 days. (See case report 2.) Proliferation of septal (perilobular) bile ducts at the boundary of two adjacent lobules. These little ducts are normally inconspicuous and resemble vascular twigs. Here, their cells are prominent, crowded together, and have large hyperchromatic nuclei. In the upper half of the photograph are shown several sinusoids with intact walls. The intervening hepatic cells have disappeared, and the lobular remnants are greatly engorged. (See also Figs. 3, 17, and 18.)  $\times 650$ . (A.I.P. acc. 126048; neg. 86332.)

FIG. 24. Duration of disease, clinically less than 1 day. (See case report 21.) Portal region. A proliferating bile duct, composed of large cells with prominent nuclei, lies diagonally across the photograph. On one side of the duct the stroma is densely infiltrated with mononuclear cells, among which may be seen a few polymorphonuclear leukocytes. The large pale cells on the opposite side of the duct are swollen histiocytes. (See also Figs. 8, 15, and 27.)  $\times 915$ . (A.I.P. acc. 123465; neg. 86347.)

FIG. 25. Duration of disease, 9 days. Inflammatory cells in the portal stroma: plasma cells, lymphocytes, and histiocytes predominate. The mass in the upper left-hand corner consists of proliferating bile duct epithelium.  $\times 1360$ . (A.I.P. acc. 126110; neg. 86331.)



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PLATE 173

FIG. 26. Duration of disease, 10 days. (See case 15.) Portal region of liver. Early proliferation of biliary ducts. The proliferating ducts have an irregular shape, due to budding and branching. The component cells are relatively large and have deeply chromatic nuclei. The stroma is densely infiltrated with inflammatory cells.  $\times 230$ . (A.I.P. acc. 111844; neg. 86340.)

FIG. 27. Duration of disease, clinically less than 1 day. Spleen. The figure shows an enlarged follicle with a prominent and partly necrotic germinal center. (See also Figs. 8, 15, and 24.)  $\times 300$ . (A.I.P. acc. 123465.)



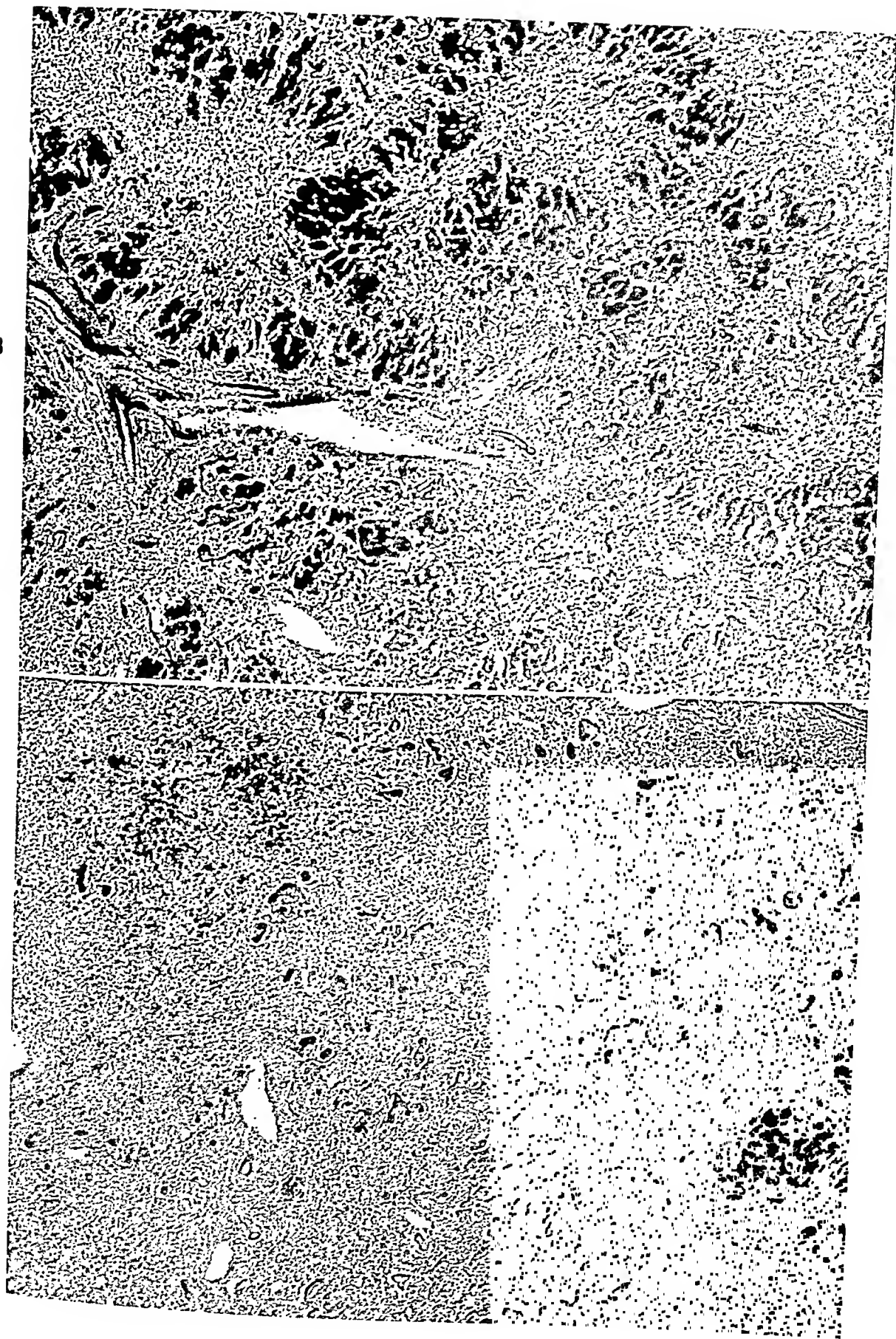
Lucké and Mallory

The Fulminant Form of Epidemic Hepatitis

PLATE 174

FIG. 28. Duration of disease, 12 days. Appearance of liver at low magnification. The photograph shows a region in which destruction of hepatic parenchyma is incomplete. A thin, irregular rim of liver cells is preserved in the peripheral zone of the lobules. The portal regions and lobular remnants are densely infiltrated with inflammatory cells. (The section photographed was cut thick in order to bring out these features.)  $\times 50$ . (A.I.P. acc. 123473; neg. 86017.)

FIG. 29. Another field from the liver shown in the preceding figure at the same magnification. In this area only occasional clumps of liver cells have been preserved. Where destruction is complete, the lobular remnants are outlined by proliferating bile ducts. The granular appearance of the interior of the lobules is the result of intense congestion and invasion by inflammatory cells. This and the preceding photograph give evidence that even in rapidly fatal cases of epidemic hepatitis involvement of the hepatic parenchyma is sometimes not uniform throughout the organ: while in large regions destruction may be complete, elsewhere patches of parenchyma are preserved. In the subacute form of epidemic hepatitis involvement of the liver characteristically is not uniform.  $\times 50$ . (A.I.P. acc. 123473.)



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PLATE 175

FIG. 30. Duration of disease, 7 days. (See case report 9.) Kidney; thick frozen section, stained with sudan. The epithelium of the neck of the proximal tubule and the convoluted segments are packed with fat. In contrast, the distal tubules, adjacent to the glomeruli, contain very little stainable fat.  $\times 230$ . (A.I.P. acc. 125602; neg. 87069.)

FIG. 31. Duration of disease, 4 days. (See case report 11.) Kidney; thick frozen section, stained with sudan. The cells of the proximal convoluted tubules are packed with fat which appears black in the photomicrograph. No fat is demonstrable in the glomerulus and very little in the adjacent loops of distal convoluted tubules. (See also Fig. 32.)  $\times 175$ . (A.I.P. acc. 126811; neg. 87068.)

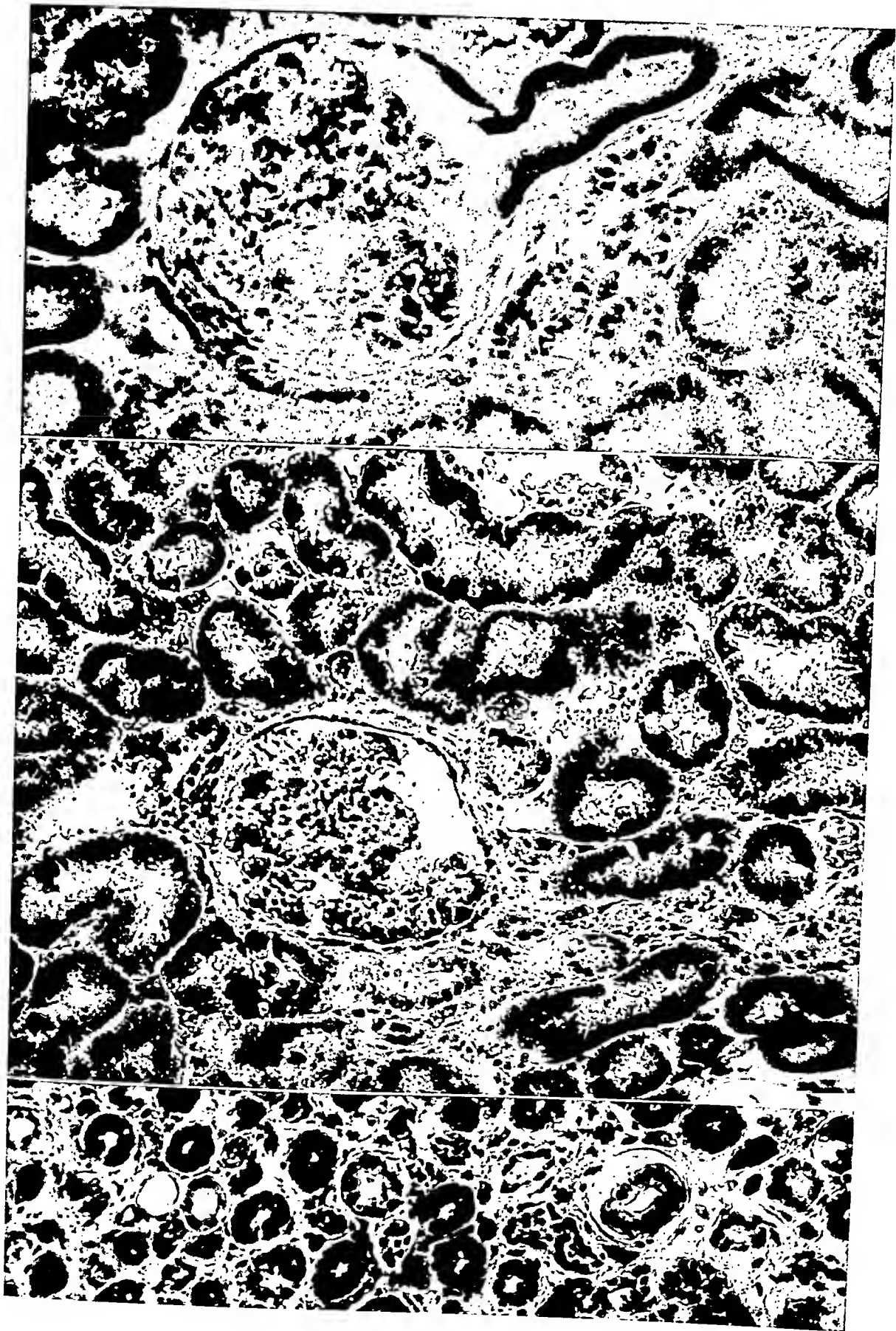
FIG. 32. Boundary zone of kidney from the same case as shown in Figure 31. Thick frozen section, sudan stain. The cells of the thick limbs of Henle are laden with fat, whereas those of the thin limbs and of the collecting tubules contain very little or no demonstrable fat.  $\times 230$ . (A.I.P. acc. 126811.)



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2







## BONE INFARCTS

### CASE REPORT WITH AUTOPSY FINDINGS \*

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University of Chicago; Chicago, Ill.)

Infarction of soft parts and especially of certain internal organs is a generally recognized condition, but infarction of bones, whether in its early or late stages, is a condition that is little known to the general medical profession. One looks in vain for more than a bare mention of this subject in textbooks on general and special pathology such as those of Kaufmann, Aschoff, MacCallum, Karsner, and Boyd. Since the publication by Kahlstrom, Burton, and Phemister<sup>1</sup> of the pathological and roentgenological findings in a series of cases of old bone infarcts, which made it possible to establish the diagnosis roentgenologically, numerous reports of cases have appeared in the literature.

The roentgenological recognition of old infarcts is based on the fact that when they are located in the heads of the femora and humeri, use leads to collapse, followed by organization of the dead bone bordering on the joints and deforming arthritis; and when they are located in the shafts and deeper portions of epiphyses, they become partly replaced by new bone and partly calcified, and cast blotchy medullary and linear peripheral shadows that are denser than those of normal cancellous bone.

The reported lesions have been confined to the long bones and have been either single or multiple. They have occurred in workers in compressed air as a sequel of the picture of caisson disease and also in an equally large number of persons, both male and female, who have never worked in compressed air and in whom the cause is usually obscure. No report has been found of bone infarction produced by nitrogen liberation from too rapid ascent in an airplane. Additional cases occurring in caisson workers and presenting the typical x-ray findings have been reported by Coley and Moore,<sup>2</sup> Walker,<sup>3</sup> Rendich and Harrington,<sup>4</sup> Bell, Edson, and Hornick,<sup>5</sup> Taylor,<sup>6</sup> and others. Of the 54 cases in Taylor's report on infarction, 13 were due to caisson disease and the remainder had no occupational history. Bell, Edson, and Hornick studied 32 compressed air workers, of whom 14 gave a history of bends, 1 of otalgia, and 4 of arthritis. Twenty-four, or 75 per cent of the number, showed typical changes of old infarction in one or more bones on x-ray examination.

\* Received for publication, July 20, 1945.

There is still a dearth of cases that have been studied pathologically. Tissue excised at operation confirmed the diagnosis in the case reported by Walker.<sup>3</sup> One of us (S. C. K.) has autopsied 3 cases in a 6-year period in a very limited service, which speaks for the relative frequency of this entity. The other<sup>7</sup> has had an opportunity to examine an infarct of the tibia obtained at autopsy and to confirm the diagnosis in case no. 4 of our original report (Part I) with Burton,<sup>1</sup> by examination of tissue since removed in an operation on the hip.

There are good reasons for the comparative silence on this subject in the literature of pathology. In the first place, many bone infarcts are entirely asymptomatic, especially for many weeks or months after their development and are therefore no cause for concern to the patient, the clinician, or the pathologist. Their discovery during life, as in the case here presented, is often entirely accidental. However, if they involve the end of the bone and lead to either collapse of the articular portion or to chronic arthritis, the pain and limitation of motion in the joints frequently lead to the establishment of the diagnosis by roentgenological examination. The striking absence or paucity of reports of infarction observed in either the early or late stages is related to the relatively infrequent examinations of the bones of the extremities at autopsy and to the infrequency with which operation gives an opportunity to obtain tissue for microscopical examination. There is every indication that if the large long bones of the body were routinely studied roentgenologically before autopsy and were sectioned longitudinally, there would be an enormous increase in the frequency of recognition of recent and old skeletal infarcts. The careful visceral necropsy usually suffices to explain the clinical manifestations and cause of death and this too often lessens the incentive to undertake the laborious task of removing the bones for further study. Roentgenograms are not taken because of the time and expense involved. The embalmer and the relatives often object to detailed skeletal examination because of mutilation of the body.

The more careful study, with sectioning of bones, of extremities amputated for arteriosclerotic gangrene would doubtless reveal evidence of blockage of blood vessels in the bones, now frequently overlooked. The departments of anatomy of medical schools have access to bones which could be routinely sectioned for gross examination, at least, without great trouble or expense. The material aid offered by x-ray examination of bone specimens in a study of this type would stimulate great interest in the correlation of the status of bone pathology with visceral pathology.

## REPORT OF CASE

While the clinical and roentgenological characteristics of the following case have been reported previously,<sup>8</sup> the subsequent verification of the bone lesions by autopsy forms the basis for this paper.

J. K., a Polish male, 55 years of age, was hospitalized at U. S. Veterans Administration, Bath, N. Y., for the sixth and final time in September, 1942, for recurrent hemorrhages from the gastrointestinal tract. The diagnoses previously established were cirrhosis of the liver with esophageal varices, secondary anemia, diabetes mellitus, arteriosclerosis, coronary and general cardiac enlargement, bilateral varicose veins, and multiple old bone infarcts.

The family history was irrelevant. The patient's only occupation had been that of chef. There was no history of exposure to compressed air. He had at times been addicted to excessive use of alcohol.

The bone lesions were discovered quite by accident, the left knee being radiographed following a minor injury in 1940. No fracture or dislocation was present, but large, irregular, mottled calcific deposits of increased density were present in the medullary portions of the distal third of the left femur and the proximal third of the left tibia. Following roentgenographic survey of the remaining skeleton, similar lesions were encountered in the distal third of the right femur and the middle third of the left humerus, the latter being but a small fleck of increased density.

The patient was rather stocky and well developed, but weak and quite anemic on this admission. The pulse was 92, respirations were 28, and the blood pressure was 94/70 mm. Hg. The physical examination further revealed a slightly enlarged liver, but was not otherwise remarkable. The stools were strongly positive for occult blood. The red blood cells numbered 2,700,000 with a hemoglobin of 45 per cent; the urine contained 1 per cent sugar while the blood sugar was 250 mg. per cent. As on many previous occasions, no source for the hemorrhage could be found in the x-ray examination of the gastrointestinal tract. Esophageal varices were looked for, but not found. The stomach was filled with a large blood clot.

The patient was given supportive treatment, including repeated blood transfusions, but continued to have hemorrhage and expired on October 21, 1942.

Autopsy disclosed a ruptured esophageal varix; cirrhosis of the liver; ascites; amyloidosis of the spleen, with perisplenitis; arteriosclerosis, coronary and generalized; cardiac enlargement (400 gm.); chronic interstitial nephritis; and an accessory kidney on the right side. The left tibia and each femur were removed. They were studied in the Laboratory of Surgical Pathology of the University of Chicago.

The external appearance of all three bones was not remarkable; the articular cartilages and synovia appeared normal. They were sectioned midcoronally. The proximal half of each femur was filled with red bone marrow, whereas the distal half contained fatty marrow except in the regions of the lesions. In the right femur, beginning 7 cm. above the lower end and extending upward for 2 cm., was a jagged, yellowish white, hard, amorphous area surrounded in most of its extent by a narrow, brownish, peripheral zone (Fig. 1). In the posterior half of the left femur, beginning 10 cm. above the lower end and extending

upward for 9 cm., the medullary content was dark gray with a mottled whitish streak of hard chalky material in the upper half (Fig. 2). Penetration with a needle revealed hard deposits in the posterior half at this level.

On section the left tibia contained yellow marrow throughout except in the upper third of the shaft. In the latter region a mottled lesion was encountered which filled the medullary canal irregularly for a distance of 10 cm. (Fig. 6). It was yellowish to gray in its mid-central portion. There were two dark brown patches, a large one superomesially and a small one inferolaterally, and a narrow dark brown zone lined the internal surface of the cortex along most of the involved segment. Roentgenograms were made of coronal sections cut approximately 0.5 cm. thick from the involved regions of the femurs (Figs. 3 and 4) and left tibia (Fig. 7). They bring out well the details of the dense mottle shadows of the lesions in the medullary canals. The slices were then decalcified and microscopical sections were made large enough to include the entire extent of the lesions. They were stained with hematoxylin and eosin. Figure 5 shows the unenlarged microscopical section of the left femur. In the upper half the marrow cavity is occupied by irregular islands of pale eosin-staining material surrounded by zones of blue-staining tissue of varying degrees of thickness. In the central portion of the lower half is a similar oblong lesion surrounded by normal appearing bone marrow. Under magnification it is seen that the pale central regions are necrotic and unorganized, while the hematoxylin-staining surrounding areas represent necrotic tissue that has been invaded by connective tissue which has become calcified in varying degrees and at the periphery has in turn been replaced by new bone or marrow.

Figure 9 shows a low magnification of the region indicated by "X" in Figure 5. Zone *a* is the outer cortex consisting of living bone which, from its normal arrangement, appears not to have been involved. Zone *b* is the inner cortex which is irregularly arranged and is composed principally of living bone and, to a small extent, of dead bone which is undergoing creeping substitution by new bone. In this region there appears to have been extensive necrosis which, for the most part, has been repaired. Calcium granules have been deposited in some of the haversian canals. At *c* is the zone of fibrous invasion of the necrotic endosteal and medullary region, with marked calcification along the inner margin. The old trabeculae are dead and surrounded by connective tissue. In the peripheral regions some of the calcified connective tissue is undergoing ossification and some of the dead trabeculae are being replaced by new bone. Zone *d* is the central necrotic

medullary region. It is, for the most part, filled with amorphous debris throughout which calcium granules are irregularly dispersed. Scattered, small, necrotic, bony trabeculae are present. Throughout the region there are vacuoles with calcium more densely concentrated at the periphery. Regions from the zones of Figure 9 are shown in detail in Figures 10 to 12. Figure 10 is from zone *b*. It shows dead bone, A, undergoing creeping replacement by new bone, B. Figure 11, from zone *c*, shows the dead trabeculae remaining, but the dead marrow is replaced by fibrous tissue, a part of which has become calcified. Figure 12, from zone *d*, shows calcium granules in the necrotic debris about vacuoles, and partly calcified connective tissue invading the periphery. In some regions the outlines of old fat cells are to be seen, with calcifying debris filling the space formerly occupied by the cytoplasm. Cholesterol slits are present in a few regions, suggesting the previous existence of hemorrhage.

The elongated island in the center of the medullary cavity in the lower part of Figure 5 was similar to the lesion above it, to which it was attached in a posterior plane as revealed by the roentgenograms. There is evidence that in places it was still being very slowly reduced in size and replaced by fatty marrow and fine bony trabeculae about the periphery. Blood vessels were relatively sparse throughout the entire section and no old obstructed vessels were seen.

Sections of the smaller lesion in the right femur showed changes identical with those of the left femur.

Figure 8 shows a microscopical section of the tibial lesion which measured 8 cm. in length and filled almost the entire medullary canal for a length of 6 cm. Its upper and lower limits are jagged and there is a small, similarly involved island 1 cm. above the mass on the mesial side of the bone. There is a bluish-staining calcified zone about its periphery which in some places borders on the cortex of the shaft and in others is separated from the shaft by a narrow zone of marrow. The contents within its calcified zone are dark brown about the periphery and gray to brown in the central region. Microscopical examination shows them to be composed of necrotic fat, partly calcified and with the outlines of many of the cells preserved, old blood pigment especially abundant about the periphery, cholesterol slits, coarse calcium granules, necrotic bony trabeculae, and invading connective tissue. Figure 13 is a low-power view extending through cortex, calcified zone, old blood pigment containing cholesterol slits, and necrotic and partly calcified marrow. Figure 14 is a higher power showing old pigment containing cholesterol slits, necrotic and partly calcified fat, and necrotic bone trabeculae. The blood vessels of the bone outside the

infarct are small and there are no signs of obliteration or of arteriosclerosis.

### DISCUSSION

The three lesions which have been described are the remains of old aseptic infarcts which have been somewhat reduced in size by connective tissue invasion, absorption, and replacement by new bone and marrow at the periphery. The reparative stimulus appears to have been long since almost completely exhausted and the unreplaced portions have undergone partial calcification and encasement by a calcified fibrous wall. There was no appreciable change in the x-ray appearance between the first examination and that at death 2 years later. The presence of much blood pigment and of cholesterol slits in the tibial lesion, especially peripherally, is evidence that it was a hemorrhagic infarct. Cholesterol slits in a few regions of the left femoral lesion are suggestive of old hemorrhage there.

The location of the infarcts suggests that they arose from blockage of the branch of the nutrient artery supplying the involved end of the respective bone. The large size of the lesions in the left femur and tibia suggests that the entire branch may have been blocked, although the anteroposterior and lateral roentgenograms reveal that the posterior portion of the bone was the more extensively involved in each case. The small size and eccentric location of the lesion of the right femur would indicate blockage of only a portion of the inferior nutrient branch. The evidences of necrosis and creeping substitution of internal cortical bone in the left femur speak for nutrient artery blockage since it supplies the internal portion of the cortex.

The exact cause of this multiple infarction cannot be stated. There was no history of work under compressed air. The structure indicates that the lesions are all of the same vintage and that they are many years old. Because of the arteriosclerosis, both generalized and coronary, one must think of vascular blockage from intimal thickening, embolism or thrombosis. The absence of infarcts in the spleen and kidneys militates to some extent against that explanation. There was no evidence of old valvular lesions. The cirrhosis of the liver should be considered since Axhausen<sup>9</sup> reported fresh infarcts in a patient dying with portal cirrhosis. The absence of recent infarcts speaks against cirrhosis as a causative factor.

### SUMMARY AND CONCLUSIONS

Autopsy studies are reported of another case of ancient infarction in multiple bones of the extremities which was diagnosed roentgenologically before death by the presence of blotchy medullary shadows

of increased density produced by calcification of the unresolved portions of the infarcts.

The exact cause of the infarction was undetermined although it may have been related to the accompanying generalized and coronary arteriosclerosis.

A review of the recent literature indicates that old calcified bone infarcts, both multiple and single, are being diagnosed frequently by the blotchy shadows and evidences of collapse and deformity of articular surfaces shown in roentgenograms.

Failure to examine the long bones routinely at autopsy is responsible for nonrecognition of a great many infarcts.

Routine roentgenography of the bones of the extremities preceding autopsy would assist greatly in the recognition of old infarcts.

A diligent routine search for bone infarcts at post-mortem examination would lead to the discovery of lesions in the early stages and help to arrive at the cause in those cases which remain unexplained.

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[Illustrations follow]



## DESCRIPTION OF PLATES

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### PLATE 176

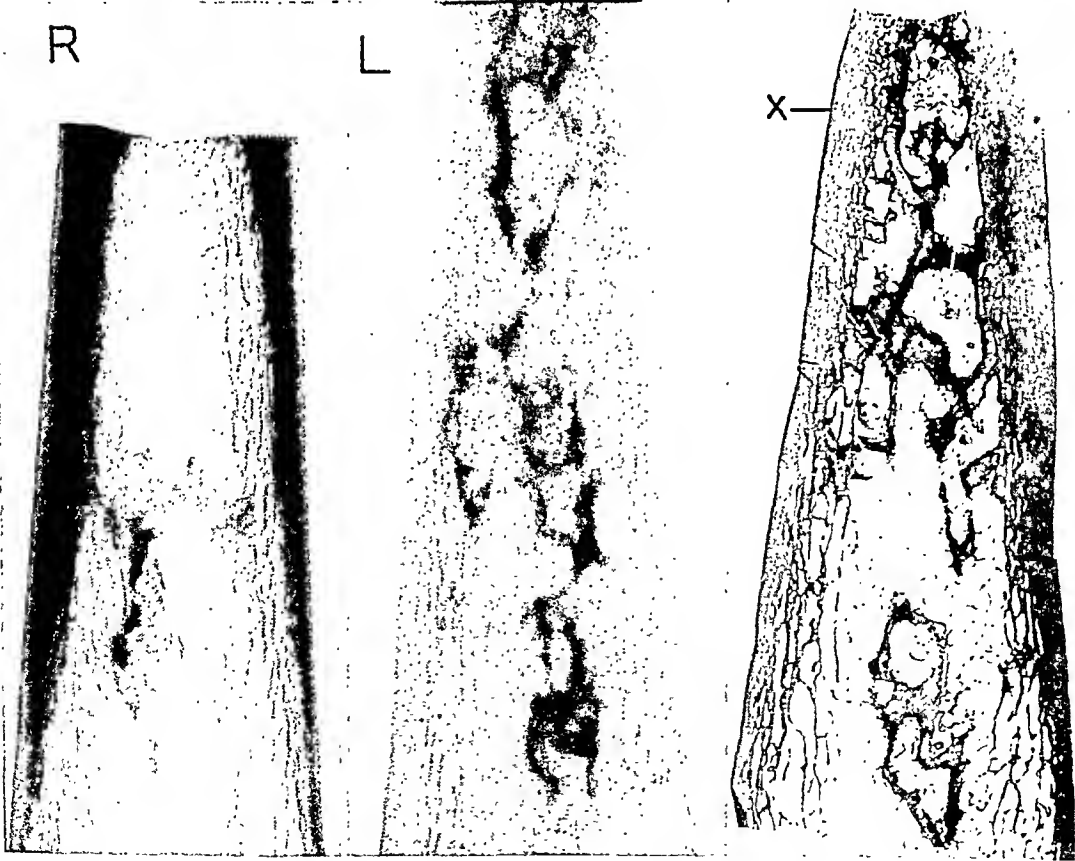
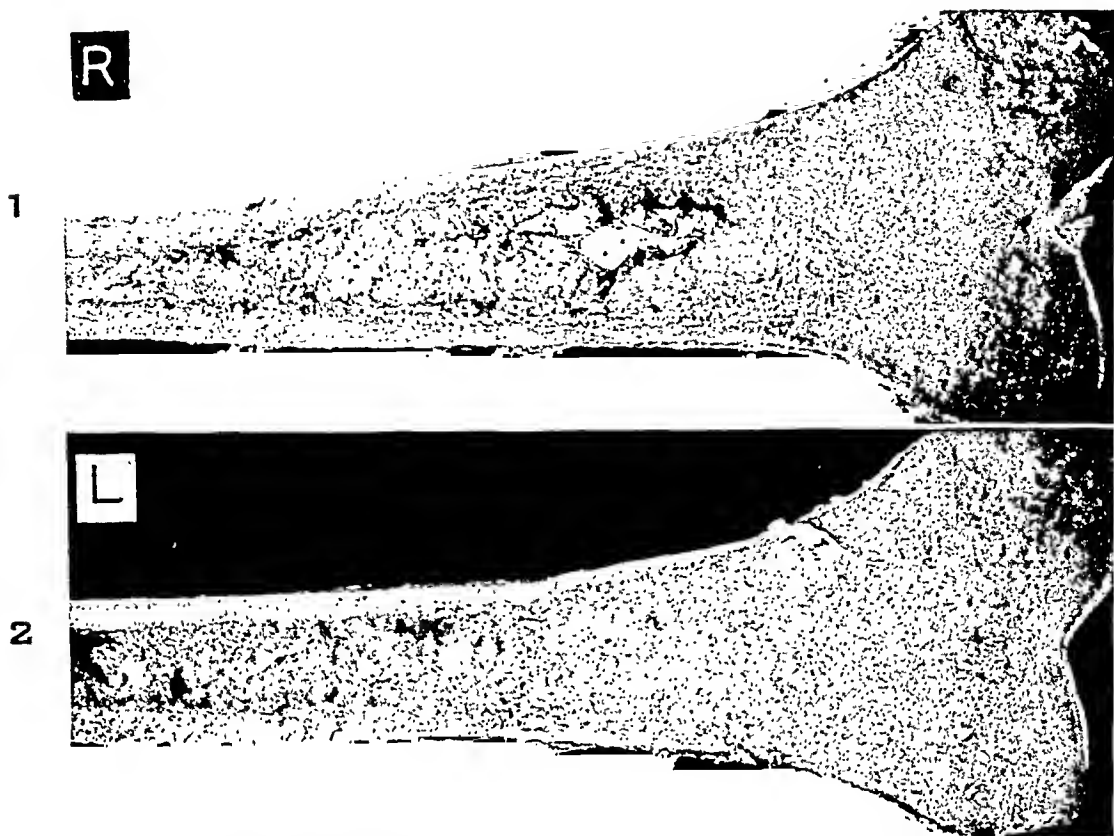
FIG. 1. Yellowish white, calcified infarct in right femur.

FIG. 2. Grayish white, calcified areas in left femur.

FIG. 3. X-ray shadow of calcified infarct in right femur.

FIG. 4. X-ray shadow of calcified infarct of left femur.

FIG. 5. Microscopical section of calcified medullary infarcts of left femur.



3

4

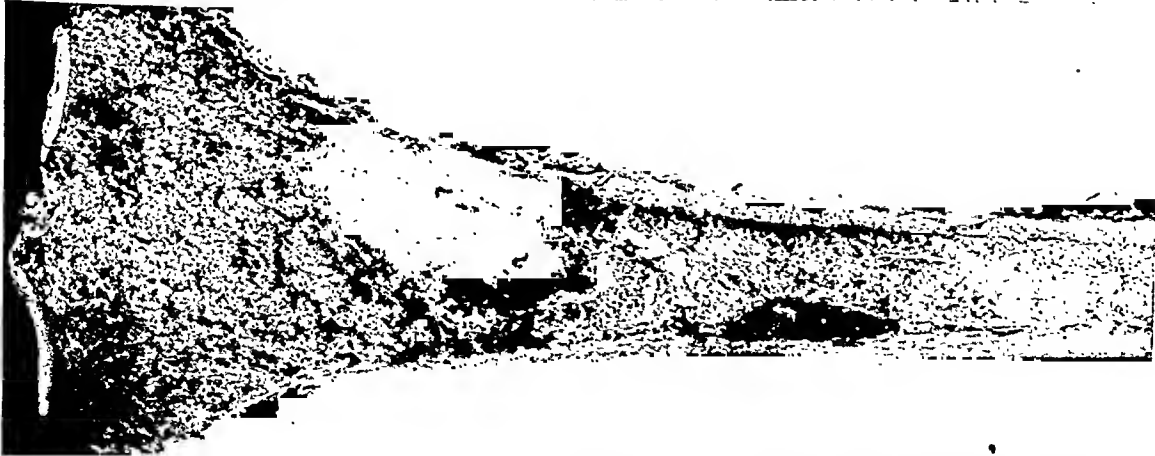
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PLATE 177

FIG. 6. Old hemorrhagic and calcified infarct of tibia.

FIG. 7. Roentgenogram of specimen illustrated in Figure 6, showing mottled increase in density of involved medullary region.

FIG. 8. Microscopical section of tibial lesion.



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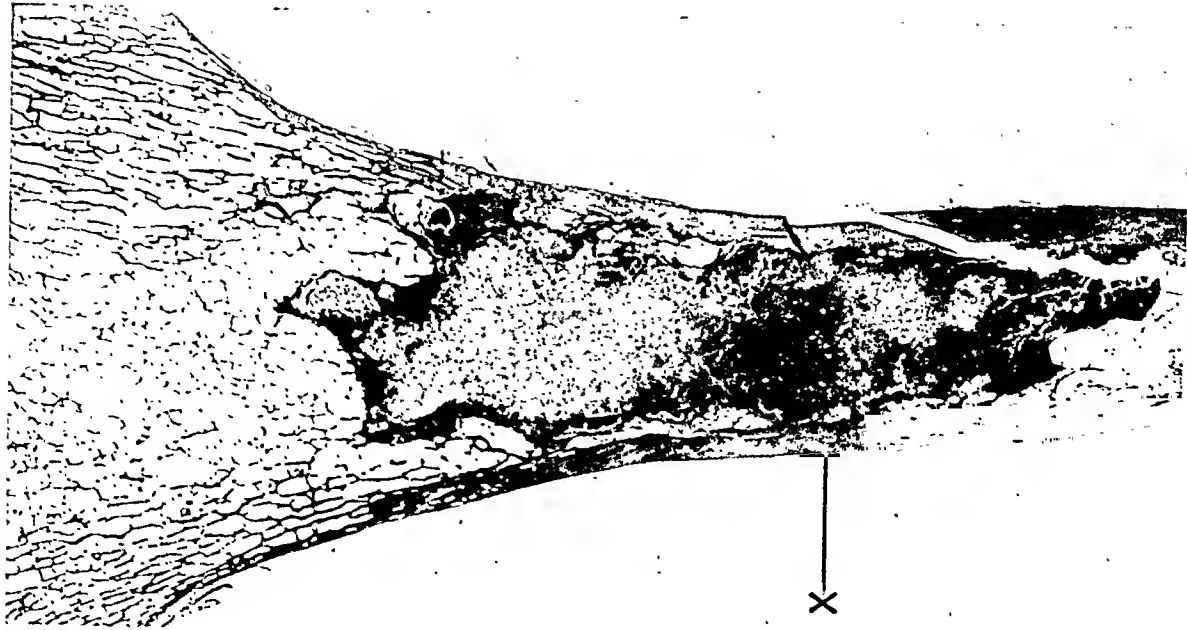


PLATE 178

FIG. 9. The are marked "X" in Figure 5 at  $8\frac{1}{2} \times$ :

- a.* Unaltered superficial cortex.
- b.* Deep cortex, necrotic, and partially replaced by new bone.
- c.* Fibrous invasion and calcification of necrotic medullary region.
- d.* Liquefaction and calcification of medullary region.

FIG. 10. Zone *b*, Figure 9, at  $190 \times$ , showing dead bone, A; being replaced by living bone, B.



a b c d

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9



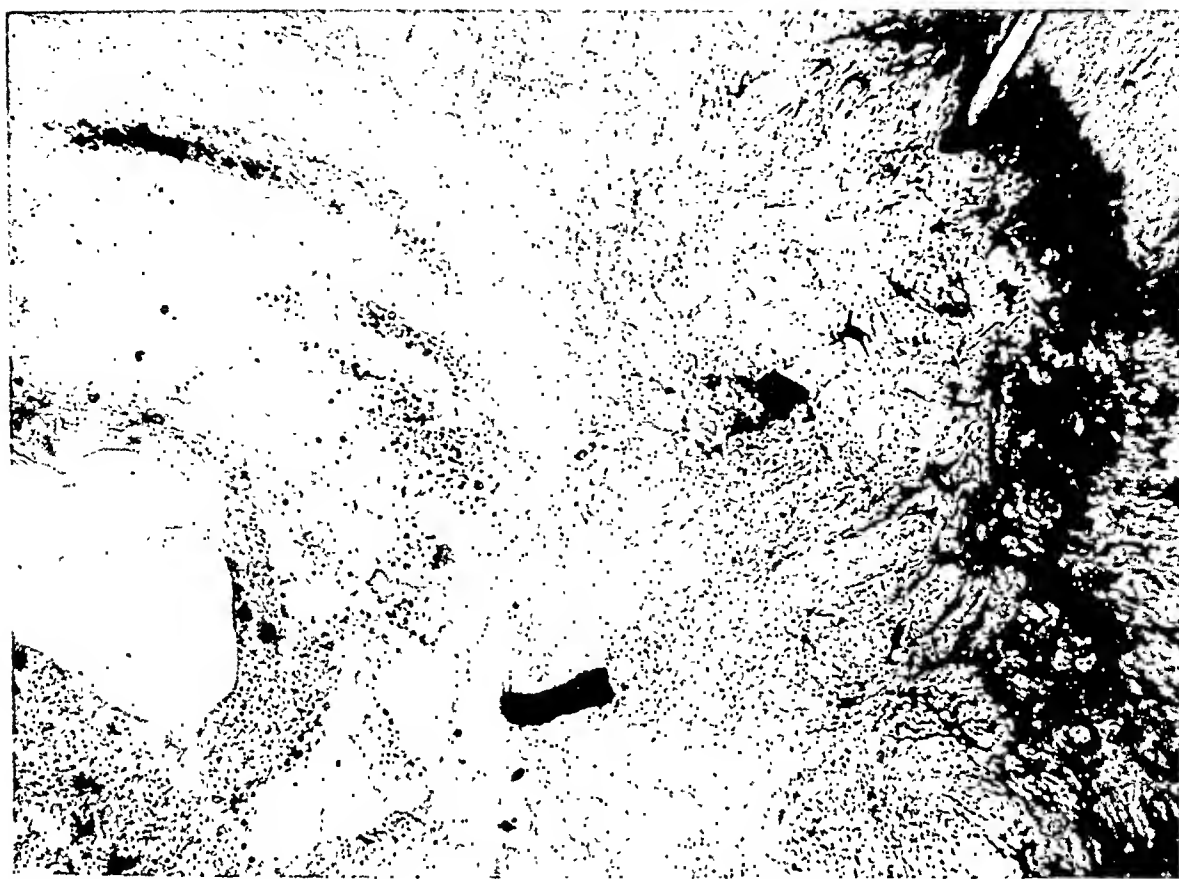
10

Bone Infarcts

PLATE 179

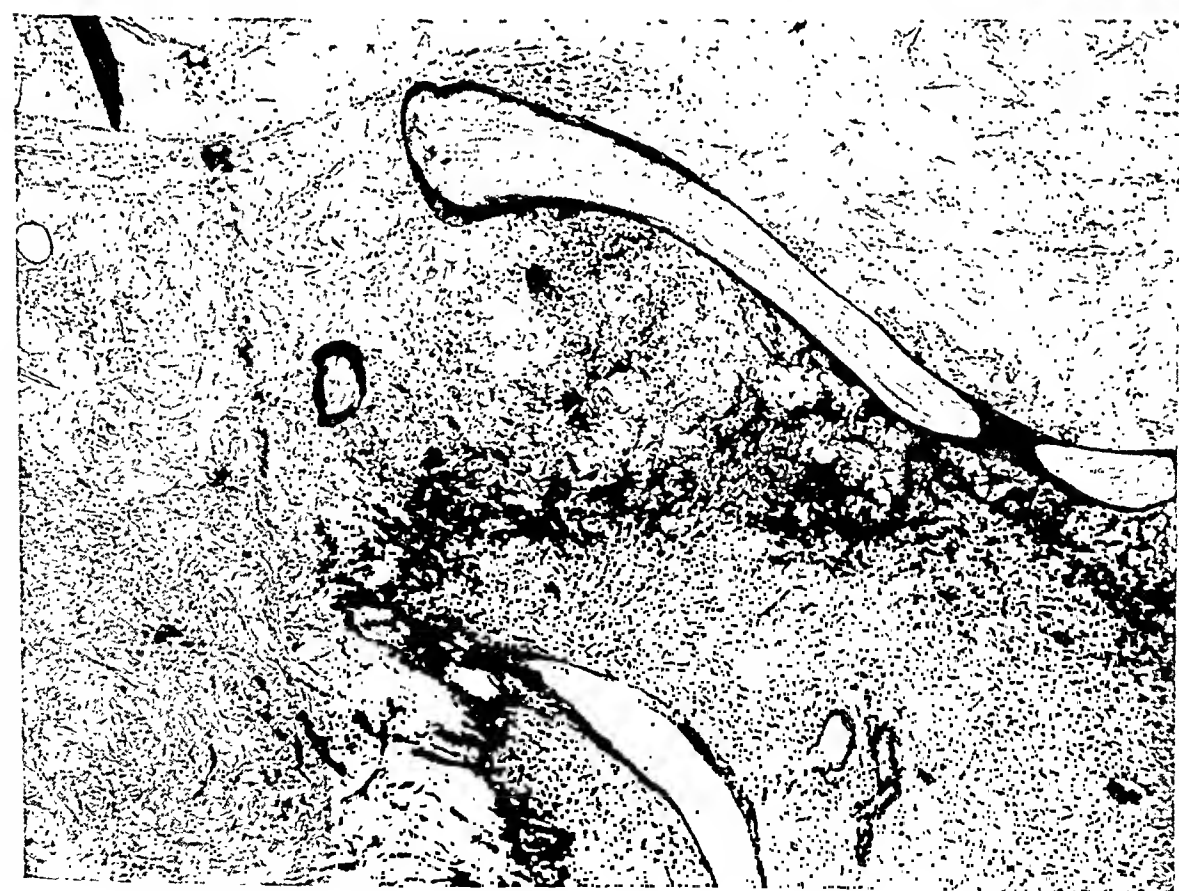
FIG. 11. Zone *c*, Figure 9, at 70  $\times$ , showing fibrous replacement and calcification.

FIG. 12. Zone *d*, Figure 9, at 45  $\times$ , showing necrosis, liquefaction, calcification, and connective tissue invasion.



12

Bone Infarcts



11

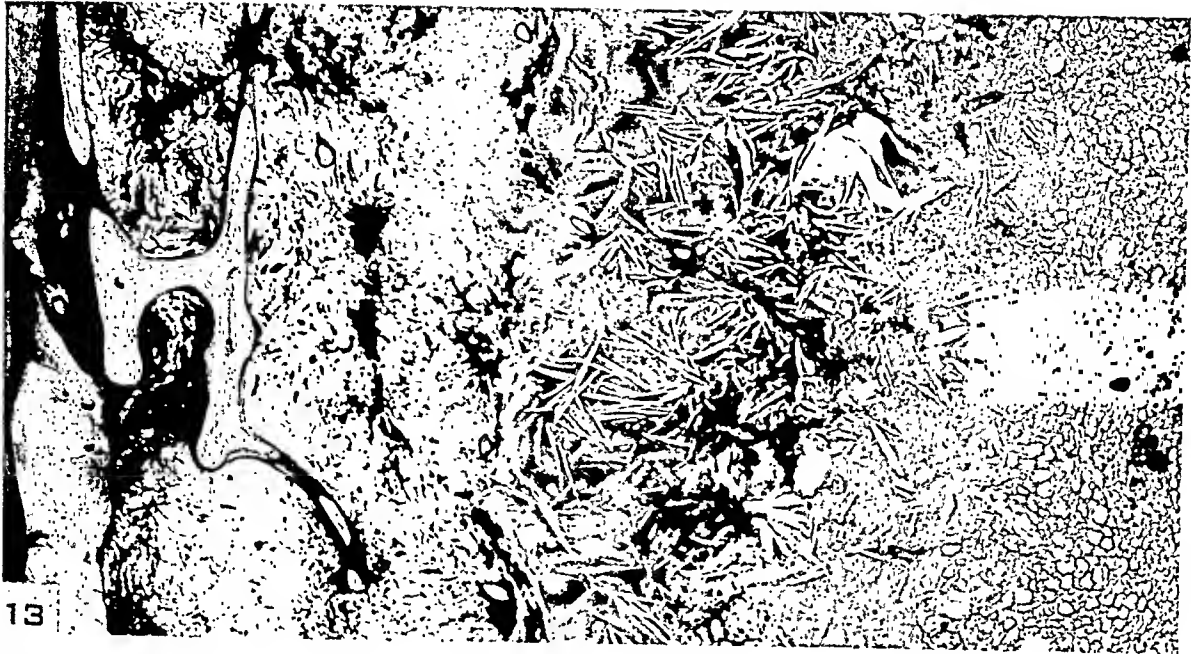
Kahlstrom and Phemister



PLATE 180

FIG. 13. Level "X" of Figure 8, at 18 X, passing through cortex, *a*; calcified zone, *b*; old blood pigment and cholesterol slits, *c*; and necrotic and partly calcified marrow, *d*.

FIG. 14. The central region of Figure 8, at 50 X, showing necrotic fat with regional calcification, *a*; and old hemorrhage and cholesterol slits, *b*.



*a* *b* *c* *d*



*a* *b*

Kahlstrom and Phemister

Bone Infarcts



## THE PATHOLOGY OF JAPANESE B ENCEPHALITIS \*

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During the second week of July, 1945, instances of an acute febrile nervous disorder began making their appearance on Okinawa-Jima in the Ryukyu group of islands. These first occurred among the native population, but sporadic cases of the disease began to appear in the U. S. military personnel stationed on the island. Increasing numbers of cases were reported during the second half of July, the level of incidence being maintained until the last week of August, when it gradually began to fall. An occasional case, only, was encountered in September. In all, there were about 30 instances of this condition in our military personnel and perhaps 200 to 300 in the natives.

Many factors contributed to the almost insurmountable difficulties encountered in determining the true incidence in the native Okinawans. In the first place, a large portion of the population was displaced from the lower to the middle third of the island, where the unavoidably crowded living conditions produced a sharp rise in the incidence of many confusing infectious diseases. Also, frequent shifting of large groups of the population from place to place necessitated the repeated parallel transfer of Military Government hospital facilities with consequent interruption of service. Then, the language barriers between the natives and the medical personnel of our Military Government all but prevented obtaining reliable clinical data. Whether because of stoicism, fear, or indifference, many of the cases among the natives, at least at first, were not reported, and, thus, it became necessary for some of our medical officers to devote most of their efforts to seeking out the natives stricken with the disease. This was time-consuming and inefficient, but undoubtedly served to locate many cases which otherwise would have gone unrecognized.

The failure to uncover some cases of this malady of the nervous system was counterbalanced by the inclusion of many instances of nervous diseases of other types. This was especially true after the U. S. Military Government Research Center E-1 was opened as the receiving hospital for such patients. On the roster of patients in this hospital were many with tuberculous meningitis, other meningitides, and malaria.

For these several reasons, accurate knowledge of the incidence of

\* The Bureau of Medicine and Surgery of the U.S. Navy does not necessarily undertake to endorse the views expressed in this paper.

Received for publication, November 29, 1945.

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the disease and its mortality rate is lacking. In view of the fact that but roughly 200 natives were involved in a population estimated at about 300,000, the nervous disorder failed to reach epidemic proportions. Endemics such as this one, thought to be due to the virus of Japanese B encephalitis, were known by certain Japanese physicians to occur in the summer months on Okinawa. For this reason, study of the present endemic was begun immediately to determine its etiology, mode of transmission, and morphologic features. Various members of the U. S. Naval Medical Research Unit No. 2, as well as the staff of the Military Government Research Center E-1, participated. This report will deal with the anatomic findings in 11 fatal cases of the

TABLE I

*Fatal Cases of Japanese B Encephalitis Listed According to Duration of Illness*

Case no.	Clinical case no.	Sex	Age	Duration of illness
			(years)	(days)
1	—	M	ca. 20	5
2	—	M	ca. 20	6
3	10	F	6	6
4	12	M	2	6
5	18	F	7	7
6	13	M	7	8
7	5	M	8	11
8	9	F	21	13
9	31	F	5	37
10	72	F	6	48
11	36	F	14	52

disease which were proved with reasonable certainty to be due to the virus of Japanese B encephalitis.

*Summary of Clinical Course.* The patients in the fatal group presented a remarkably uniform clinical picture.\* In each instance where this information is available (8 of the 11 cases), the onset of the illness was sudden with fever and headache. In addition, 3 of the patients became somnolent, lethargic, or drowsy and 2 had generalized convulsions. Prominent among the neurologic signs in the course of illness were nuchal rigidity, positive Kernig's sign, stupor, loss of speech, generalized convulsions, flaccid paralysis, Babinski's sign, and absent abdominal reflexes. Three of the patients had hyperreflexia, 2 had trismus, and at least 2 others had dissociated ocular movements. Three of the patients who survived more than 30 days gradually became spastic, were comatose, and lapsed into a peculiar "vegetative" state.

In Table I are listed the cases in chronologic sequence according to

\*I am indebted to Lt. Comdr. Leon Lewis, M.C., U.S.N.R., Lt. Comdr. Lewis Thomas, M.C.(S), U.S.N.R., and Lt. John L. Peck, M.C.(S), U.S.N.R., for the clinical records of these cases.

the duration of illness. The first patient was a private in the U. S. Army and the second was a sergeant in the U. S. Marine Corps Reserve. These 2 represented the only fatal cases in military personnel. The remaining 9 patients were native Okinawans. All patients who died were under 25 years of age.

*Complement-Fixation Tests.* The presence of complement-fixation reactions with the virus of Japanese B encephalitis and the sera from cases 9, 10, and 11 was demonstrated by Lt. Comdr. Horace L. Hodes, M.C.(S), U.S.N.R. On the 5th day of illness, the serum from case 9 had no complement-fixing properties, but on the 19th day the complement-fixation test was positive (4 plus) in a dilution of 1:16 and weakly positive (1 plus) in a 1:32 dilution. Two days before death, however, the test again became negative. The serum from case 10 was weakly positive (1 plus) for complement-fixation in a dilution of 1:64 on the 9th day of the disease, but it was strongly positive (4 plus) in a dilution of 1:124 on the 31st day of illness. The complement-fixation test with the serum from case 11 was negative on the 3rd day of the disease, but on the 32nd day a positive reaction (3 plus) was obtained in a dilution of 1:32. These findings indicated that the nervous disorder to which the patients listed in Table I succumbed was caused by the virus of Japanese B encephalitis.

#### ANATOMIC CHANGES IN THE NERVOUS SYSTEM

The nervous systems and other viscera of all but 4 of the fatal cases were removed at necropsy by several different pathologists, sectioned, placed in 10 per cent neutral formaldehyde, and sent by air to the Laboratory of Pathology of the U. S. Naval Medical Research Unit no. 2 on Guam. I returned from Okinawa with the material from the 4 remaining cases, and the preparation of microscopic slides of all the tissues was begun in our laboratory. Special attention was devoted to the nervous system, and a variety of different staining methods was employed for the demonstration of ganglion cells, glia, medullary sheaths, axis cylinders, and fat.

#### *Macroscopic Findings*

The nervous tissue and meninges of the patients who died in less than 2 weeks of illness disclosed but few findings on macroscopic examination. The meninges were not clouded by exudate and none contained an excess of cerebrospinal fluid. Congestion of the pial vessels was noted, but was not extreme. In several cases, small foci of congested blood vessels and clusters of petechiae were found in the cerebral cortical gray matter and in the basal ganglia. Such vascular

lesions, however, were neither extensive nor constant as to location. In two of the spinal cords, the central gray matter of the cervical region was discolored as in acute poliomyelitis. On the whole, however, the changes found in the acute cases on naked-eye examination were unimpressive.

In the 3 patients who survived more than a month, cerebral lesions were easily observed and were of rather unusual types. Whereas, again, the pia-arachnoid and the meningeal vessels were not remarkable, the cortical gray matter of both cerebral hemispheres was altered in a characteristic fashion. Patches of different size, sometimes pale because they were apparently acellular and at other times finely granular and distinctly gritty, were found in the cortical gray matter of every lobe and nearly every gyrus. A sensation as if cutting through calcified tissue was produced when the brains were sectioned. These lesions had no predilection for either the troughs or the crests of the gyri. Similar but larger lesions were found in the central gray matter, where the pallidal nuclei, the thalami, the red nuclei, and the substantiae nigrae appeared to be involved selectively. In addition, cystic changes resembling encephalomalacia were seen both in the pallidal nuclei and the substantiae nigrae. In the pontile and dentate nuclei of the cerebellum there were faintly visible, minute foci of brown discoloration. Close examination of the cerebellar folia disclosed a peculiar blurring of the cortical pattern which was seen to a lesser extent also in one or two of the more acute cases. The diameters of the spinal cord of case 10 appeared to be decreased, and it was only with great difficulty that the outlines of the central gray matter could be visualized. This, again, was reminiscent of the changes in poliomyelitis.

### *Microscopic Findings*

Microscopic examination of the nervous systems of these patients revealed a great variety of lesions. In the more acute cases the leptomeninges were infiltrated with sparse lymphocytes, which were somewhat more numerous in the depths of the cerebral sulci (Fig. 1). A few large mononuclear cells and macrophages were also found in the pia-arachnoid of the older cases, but the cellular exudate was never a very conspicuous feature. Also, as noted in the macroscopic description of the acute cases, foci of capillary congestion and "ring" hemorrhages were present in the basal ganglia (Fig. 2). Perivascular "cuffing" by lymphocytes and, on occasion, by monocytes and macrophages (some containing fat) was present in many different parts of the brain, including the white matter, where other types of pathologic change were absent (Fig. 3). Small round cells were found not only in the

Virchow-Robin spaces but also in the vascular sheaths. The perivascular inflammatory process was most evident in the spinal cord.

Far more noteworthy than the exudative reaction was the widespread neuronal destruction that occurred in the cerebral cortex, the basal ganglia, the pons, medulla, cerebellum, and spinal cord. The degree of neuronal involvement varied from case to case and from zone to zone in each case. The essentially degenerative changes in the ganglion cells were sometimes focal in extent, limited to small groups of these cells, as seen in the cerebral cortex illustrated in Figure 4, and sometimes involved all the cells of a specialized region, as in the cornu Ammonis formation (Fig. 5). The injured cells were often only 2 or 3 in a group, or as many as 20 or 30. Various types of cellular disintegration were noted. There were simple chromatolytic changes as well as the "severe" cellular change of Nissl. Many involved ganglion cells were shrunken and pyknotic, and some were eosinophilic. A few had pericellular incrustations, the pericellular spaces around others were widened, and some had become completely autolyzed and had disappeared. Where this involved larger groups of neurons, the affected areas had a characteristic spongy appearance.

The neuronal lesions were at times accompanied by the appearance of infiltrations of neutrophilic leukocytes as well as by proliferations of microglia (Figs. 6 and 8). Where the leukocytic response was excessive, the focal lesion assumed the characteristics of a miliary abscess, although rarely was the associated tissue necrosis of such extent as to suggest much suppuration. In the anterior horns of the spinal cord the glial and leukocytic reactions duplicated the histologic picture of acute poliomyelitis (Fig. 8). The injured anterior horn cells were invaded by both leukocytes and microglia in the process of neurophagia (Fig. 7). The latter was rarely seen in regions other than the cord; elsewhere the damaged ganglion cells were indicated by increased glial satellitosis. Glial nodules as well as infiltrating lymphocytes, mononuclear cells, and polymorphonuclear leukocytes, similar to the reaction noted in the cord, were found in the caudate nuclei, the pallida, the red nuclei, the inferior olives, the cranial nerve nuclei in the floor of the medulla and, especially, in the substantia nigra (Fig. 9). As the melanin-containing ganglion cells of the latter region disintegrated, dark brown pigment granules were found scattered in the interstitial tissue. Just as in the cerebral cortex, when large numbers of neurons disappeared from the substantia, a spongy condition developed in this structure (Fig. 10).

The older lesions in both the cortical and central gray matter of the cerebrum developed a more complex histologic picture by the addition



of numerous macrophages (Figs. 11, 12, and 13). They still remained rather circumscribed, and the presence of some leukocytes coupled with necrosis of the interstitium conferred on them simultaneously certain features of encephalomalacia and also of abscess formation. The mononuclear phagocytes contained but sparse amounts of lipoid material. Most frequently these lesions were unrelated to blood vessels, but occasionally such a relationship did exist even if it appeared to be fortuitous (Fig. 12). In none of the cases were cerebral vascular occlusions found, although in one (case 11), a patient who developed a terminal necrotizing pneumonia, many cerebral vessels contained "leukocytic thrombi." It is emphasized here that these lesions were confined to the gray matter and were never seen in the white matter of any part of the nervous system.

There was still another type of commonly encountered lesion, the most characteristic features of which were its widespread dissemination, its circumscribed, focal appearance, and its almost complete lack of accompanying cellular fixed-tissue and exudative response (Figs. 14 to 20). Were it not for the fact that these changes were also limited to collections of ganglion cells, they could easily be mistaken for the lesions of multiple sclerosis. They involved the white matter only by extension from the gray, as occurred occasionally in the pons (Figs. 16 and 19). Even here, however, they were often quite sharply limited to the pontile nuclei, leaving the intervening medullary tracts unaffected (Fig. 20). Also, as sometimes occurred, when the lesion occupied a position in the sixth layer of the cerebral cortex, the subjacent white matter was spared (Fig. 17). Myelin sheath stains of such foci of injury disclosed an almost complete absence of fibers, with small local accumulations of fatty droplets. In sudan III preparations, more fat was demonstrated within phagocytes in the perivascular sheaths of the regional blood vessels. Ballooning, ribbon formation, and fragmentation of dendrites and axis cylinders were demonstrable with the Trelles\* and Bielschowsky methods of staining (Fig. 18). Essentially none of these lesions was around or related to blood vessels. In many of the lesions the injured, disintegrating ganglion cells were still visible (Figs. 19 and 20). In some a feeble response on the part of the microglia and small round cells was in evidence (Figs. 16 and 17). In others, the disappearance of ganglion cells and interstitial glia left a barren field of delicate but disrupted fibrils. The fate of such lesions could be seen in the patient who survived 52 days (case 11), in whom their contraction by gliogenous scarring was evident in the injured pontile nuclei (Fig. 21).

\* Trelles, J. O. Technique d'imprégnation argentique pour les coupes à la celloïdine. *Rev. neurol.*, 1932, 1, 459-460.

The peculiar cellular architecture of the cerebellar cortex in part accounted for the type of lesion seen in this structure. As elsewhere in the nervous system, the primary injury was to the ganglion cells, the Purkinje cells being more involved than the small granular cells. Along the dendritic processes of the former cell type, in the molecular layer, glial "lawns" appeared (Figs. 22 and 23). These were the invariable indication that the Purkinje cells were severely damaged, and they were present as often in the depths as in the crests of the cerebellar folia. They were the counterpart of the lesions seen in the cerebral cortex and illustrated in Figure 11. The second type of lesion encountered, namely, the spongy transformation of the Purkinje and molecular layers (Fig. 27), also had its complement in the cerebral cortex, basal ganglia, and pons (Figs. 14 to 20). These spongy areas were practically acellular and contained a fine fibrillary structure which in part was composed of degenerated dendrites (Fig. 24).

As noted macroscopically, the 3 patients in this series who survived longest had widely distributed deposits of calcium in the cerebral cortex and basal ganglia. In some regions, the particles of calcium salts were bunched in small patches (Fig. 25), while in others, such as the cerebral cortex, they replaced all the laminae in long, wide bands (Fig. 28). Von Kossa's silver nitrate method of staining proved that the essential nature of the fine, gritty particles in the tissue was calcium salts (Fig. 26). The coarser calcareous granules were found at sites where the preceding parenchymal injury was extensive enough to have resulted in large zones of encephalomalacia or in cystic degeneration (Figs. 29, 30, and 31). Evidently with time, the deposition of inorganic salts was added to and formed increasingly larger accumulations (Figs. 32 and 33). The walls of venules and arterioles became calcified, as was noted in children of 5, 6, and 14 years, respectively. In the wake of the deposition of calcium salts, an active foreign body giant cell response appeared. Particles of calcium salts were very often found engulfed by the multinucleated cells (Figs. 34 and 35). Also, numerous microglia, transformed into large mononuclear phagocytes, contributed their effort towards the removal of this material.

The ultimate outcome of certain of the lesions in the nervous system was either demonstrated in the more chronic cases or could be surmised. Mention has already been made of the reparative process initiated by the glia, chiefly fibrillary astrocytes, at the sites of focal encephalomalacia and cystic degeneration. The larger degenerated zones, after the débris was removed by phagocytosis, remained as cysts quite similar to those so often resulting from cerebral hemorrhage or infarction. It seems doubtful that much of the calcium salts which were deposited in the chronic lesions could ultimately be removed by phagocytosis.

Rather, it would seem that in nonfatal cases such lesions would, in time, be encapsulated either by fibrosis or fibrillary gliosis, or by both processes acting together towards the same accomplishment. What change followed the destruction of individual ganglion cells, which occurred throughout the brain but was best typified by the Purkinje cells, is illustrated in Figure 36. Here, a cerebellar folium is shown in which not a single Purkinje cell remained. This was characteristic of many of the cerebellar folia in all three chronic cases. It was noted that nothing remained of the glial "lawns" seen in the molecular layer of the acute cases. The Bergmann layer of glia showed no attempt at replacement gliosis, which often masks the loss of Purkinje cells. There was, also, a general diminution in the number of neurocytes in the granular layer of the cerebellar cortex.

#### ANATOMIC CHANGES IN OTHER ORGANS

Organs other than the nervous system were available for detailed microscopic study from 3 of the acute cases (nos. 3, 5, and 7) and 2 of the chronic cases (nos. 9 and 11). Each of the 3 acute cases had typical bronchopneumonia of a noninterstitial type which was not suggestive of a virus etiology. This was the only lesion found; the other viscera were normal.

The patient who lived for 37 days after the onset of encephalitis (case 9) had an organizing pneumonia suggestive of a pneumococcal process. She, too, failed to show those changes which are associated with virus pneumonia. Her other organs contained no anatomic changes. The last patient (case 11) had a necrotizing pneumonia due to aspiration but, in addition, there were old caseous tuberculous lesions in parts of both adrenals. Ulceration of the gastric mucosa with underlying caseation and Langhans' giant cell formation were also present. Acid-fast organisms were demonstrated in both the adrenals and gastric ulcers. Tuberculous changes were not found in any other viscera, including the mediastinal and mesenteric lymph nodes.

#### DISCUSSION

The histopathologic changes in 11 cases of widespread nonsuppurative encephalomyelitis were unlike those of the post-vaccinal and the post-infectious encephalitides. In the latter, the lesions are confined almost exclusively to the central white matter of the brain and the fiber tracts of the spinal cord. They are almost always perivascular in distribution and consist essentially of a breakdown of the medullary sheaths, accompanied by monocytic inflammatory changes. In the present cases, the white matter was spared except in those rare in-

stances where the lesions spread from affected collections of ganglion cells to encroach on the myelinated fibers. But primarily, the disease was one of injury to ganglion cells with secondary inflammatory, and regressive glial, changes. Certain of its more distinctive features, such as the acellular plaques in the cerebral cortex and basal ganglia, the "spongy" lesions in the cerebellar cortex, and the deposition of calcium salts in the chronic cases, conferred an individuality upon it, but it still resembled other forms of encephalitis of virus etiology. These are encephalitis lethargica (von Economo's disease), St. Louis encephalitis, and the American equine encephalomyelitides. It also has similarities to the now presumably extinct Australian-X disease and the common disease of sheep in Scotland known as louping ill.

A number of workers have previously described the pathology of Japanese B encephalitis. All have called attention to the perivascular cuffing with lymphocytes and the focal infiltrations of mononuclear cells in the meninges. Degenerative changes were noted in the nerve cells of the basal nuclei, substantia nigra, pons, and medulla, and small foci of necrosis were described in these structures. Bertrand and Miyashita<sup>1</sup> described definite plaques of demyelination in some of their cases. Presumably, these lesions were like those portrayed in the present communication, confined to the cerebral and cerebellar cortical gray matter and the basal ganglia, and were not like the lesions of post-infectious encephalitis previously mentioned, or of multiple sclerosis. Diffuse polymorphonuclear leukocytic infiltration was noted by Kingo<sup>2,3</sup> in the cerebrum, basal ganglia, and spinal cord. Kaneko and Aoki<sup>4</sup> drew attention to the formation of abscesses and widespread destruction of ganglion cells in this form of encephalitis. Other valuable contributions to the pathology of this disease have been made by Flexner,<sup>5</sup> Hashimoto, Kudo, and Uruguchi,<sup>6</sup> and Pette.<sup>7</sup> No one, however, has called particular attention to the outstanding destruction of Purkinje cells, the formation of glial "lawns" and "spongy" changes in the molecular layer of the cerebellar cortex, and the widespread deposition of calcium salts with its attendant foreign body response in the chronic cases.

It is fortunate that uncontroverted evidence is at hand to prove that the nonsuppurative encephalomyelitis described in this report is due to the virus of Japanese B encephalitis. This evidence, in the form of complement-fixation tests, was supplied by Hodes, Thomas, and Peck.<sup>8</sup>

#### SUMMARY

The histopathologic findings in 11 cases of Japanese B encephalitis are presented; they may be summarized as follows:

There was focal as well as widespread destruction of ganglion cells in the cerebral cortex, basal ganglia, substantia nigra, red nuclei, cranial nerve nuclei in the floor of the fourth ventricle, inferior olives, cerebellar cortex, dentate nuclei, and cornua of the spinal cord. These destructive changes sometimes occurred alone, but were often accompanied by lymphocytic and polymorphonuclear leukocytic infiltration and by microglial proliferation. When these cellular reactions were associated with interstitial necrosis and phagocytosis by macrophages, focal lesions resulted which, on the one hand, resembled miliary abscesses and, on the other, patches of encephalomalacia.

In the cerebral cortex the injured ganglion cells were surrounded by increased numbers of glial satellites; in the cornua of the spinal cord there was neuronophagia.

The cerebrospinal meninges were usually infiltrated with small numbers of lymphocytes and monocytes. Perivascular collars of lymphocytes were present in the nervous parenchyma.

A few of the acute cases had capillary congestion and focal "ring" hemorrhages in the basal ganglia.

"Lawns" of glia appeared in the molecular layer of the cerebellar cortex at sites of Purkinje cell injury.

Acellular plaques were seen in the cerebral cortex, basal ganglia, and cerebellar folia. In these, there was destruction of the medullary fibers, dendrites, and axones. They had a spongy appearance and resembled the plaques of multiple sclerosis, except that they were confined to gray matter.

A reparative astrocytic gliosis occurred at the sites of smaller destructive lesions in the chronic cases.

The larger zones of encephalomalacia persisted as cyst-like lesions.

Focal and diffuse deposition of calcium salts took place at sites of injury in the chronic cases. These deposits stimulated a foreign body response with multinucleated giant cell formation.

The other viscera failed to show uniform or characteristic pathologic changes.

Acknowledgment is made of the valuable assistance of Robert H. Jackson, Pharmacists' Mate, 2nd Class, U.S.N.R., in histologic technic, and of Roy W. Poe, Pharmacists' Mate, 1st Class, U.S.N.R., in photography.

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8. Hodes, H. L., Thomas, L., and Peck, J. L. Cause of an outbreak of encephalitis established by means of complement-fixation tests. *Proc. Soc. Exper. Biol. & Med.*, 1945, 60, 220-225.

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[ Illustrations follow ]

## DESCRIPTION OF PLATES

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All photomicrographs were made from preparations stained with hematoxylin and eosin, unless otherwise indicated.

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### PLATE 181

FIG. 1. Case 7. Lymphocytic infiltration in cerebral meninges. Membrana limitans not invaded. Mild astrocytic proliferation in molecular layer.  $\times 100$ .

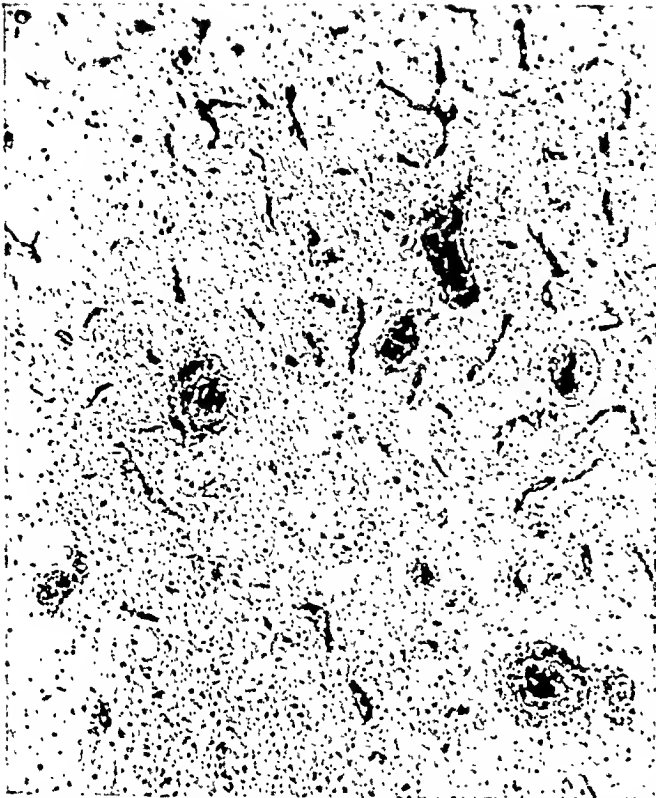
FIG. 2. Case 4. Capillary congestion and "ring" hemorrhages in basal ganglia.  $\times 100$ .

FIG. 3. Case 7. Perivascular "cuffing" by lymphocytes in anterior horn of spinal cord.  $\times 350$ .

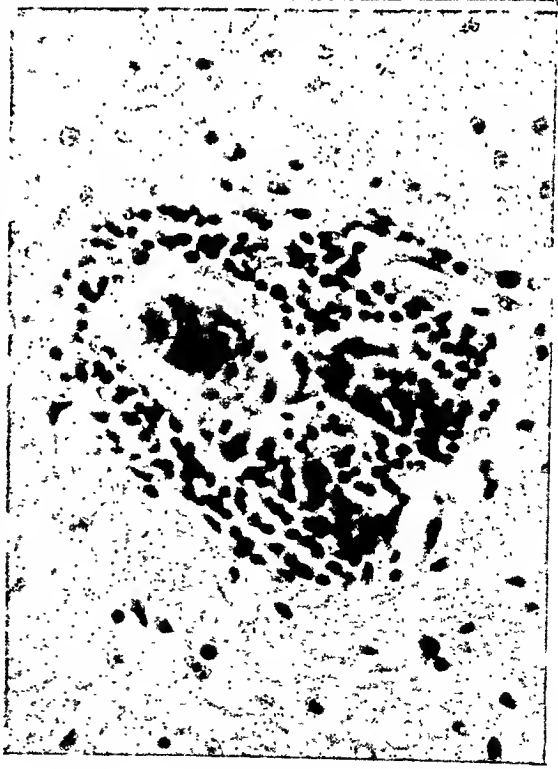
FIG. 4. Case 5. Focal degenerations of ganglion cells in cerebral cortex.  $\times 100$ .



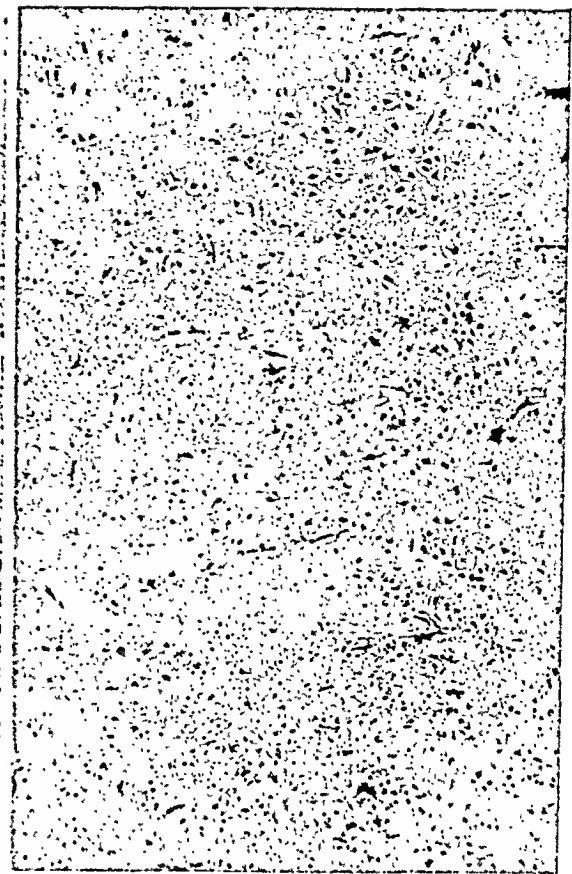
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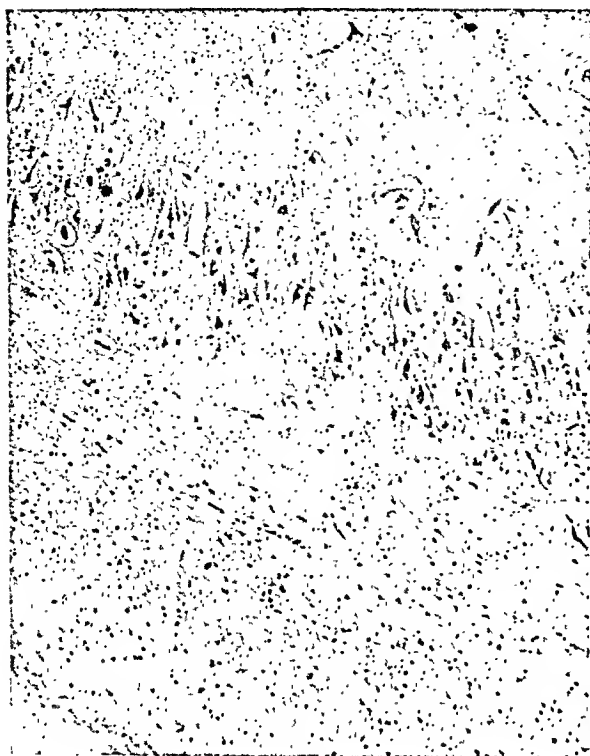
Zimmerman

Japanese B Encephalitis

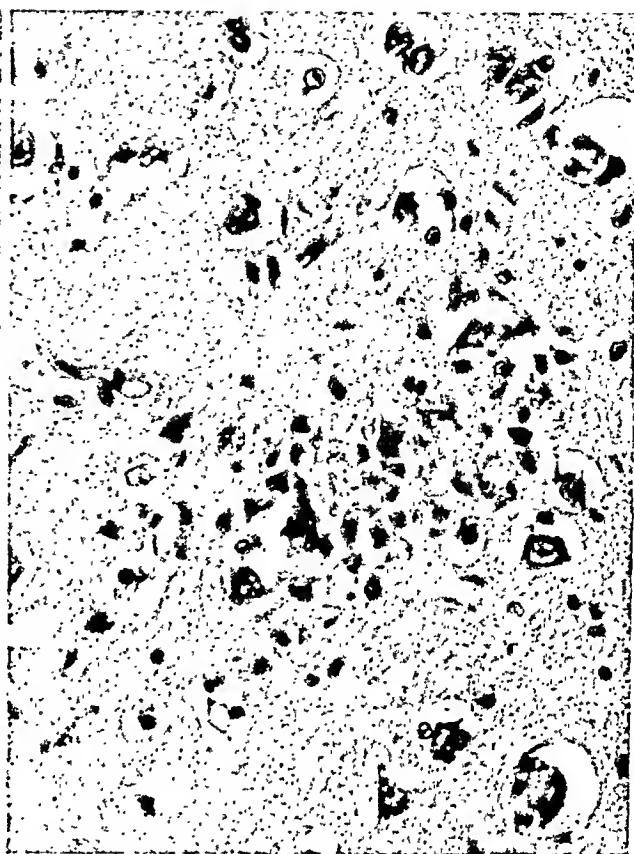


PLATE 182

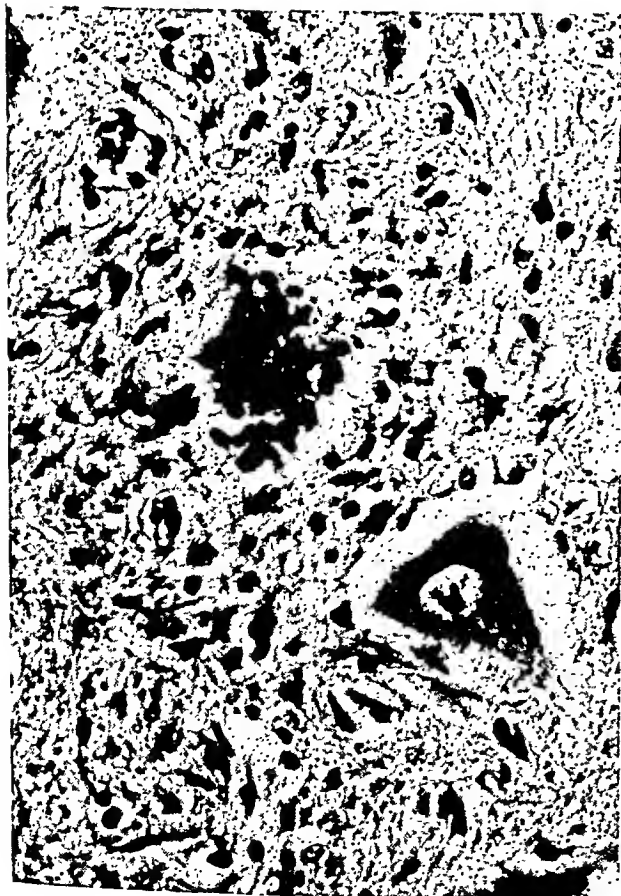
- FIG. 5. Case 3. Degenerative changes in all ganglion cells of Sommer's sector of the cornu Ammonis formation.  $\times 100$ .
- FIG. 6. Case 2. Focus of neuronal disintegration in cerebral cortex, with lymphocytic, polymorphonuclear leukocytic, and microglial response.  $\times 350$ .
- FIG. 7. Case 2. Neuronophagia of anterior horn cell of spinal cord.  $\times 350$ .
- FIG. 8. Case 2. Glial and lymphocytic reaction in anterior horn of spinal cord. Of note is the similarity to lesions in acute anterior poliomyelitis.  $\times 100$ .



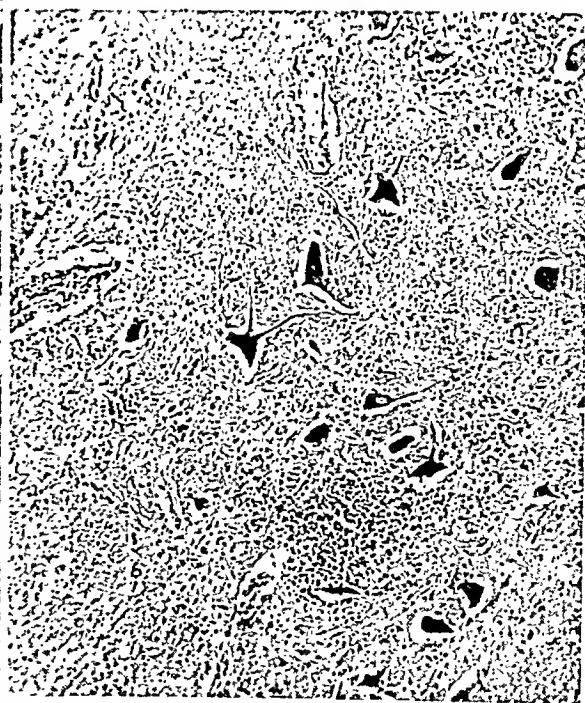
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Japanese B Encephalitis

PLATE 183

FIG. 9. Case 2. Nonsuppurative inflammatory reaction in substantia nigra.  $\times 100$ .

FIG. 10. Case 2. Disappearance of melanin-containing neurons and "spongy" formation in substantia nigra.  $\times 100$ .

FIG. 11. Case 5. Miliary abscess-like lesion in cerebral cortex. Macrophages, leukocytes, and interstitial necrosis. No relationship to blood vessels.  $\times 100$ .



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PLATE 184

FIG. 12. Case 8. Lesion in basal ganglia similar to that illustrated in Figure 11, but related to venule.  $\times 100$ .

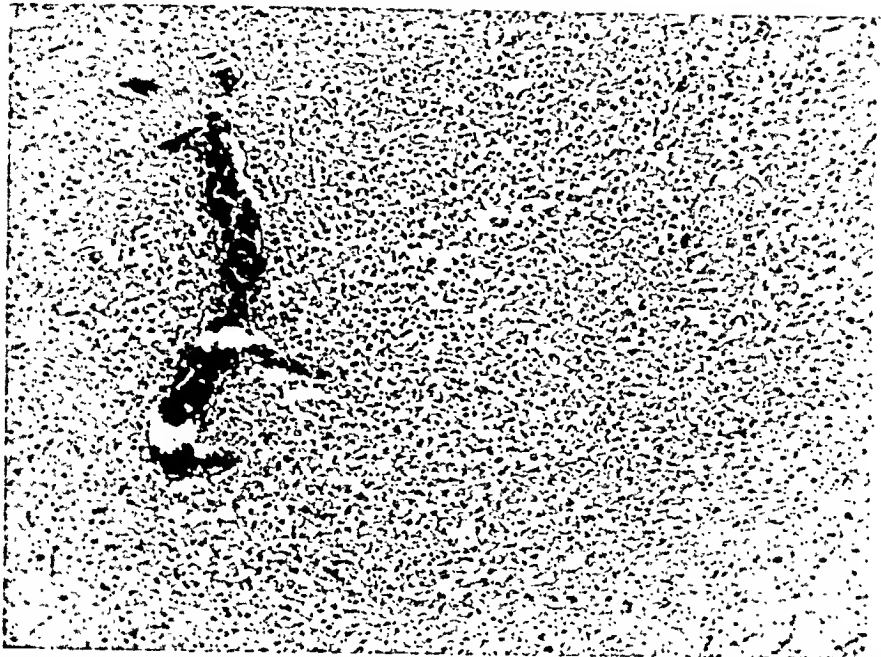
FIG. 13. Case 4. Focal, nonsuppurative reaction in red nucleus.  $\times 100$ .

FIG. 14. Case 5. Focal, acellular lesion in cerebral cortical gray matter. Superficially, like plaque in multiple sclerosis.  $\times 100$ .

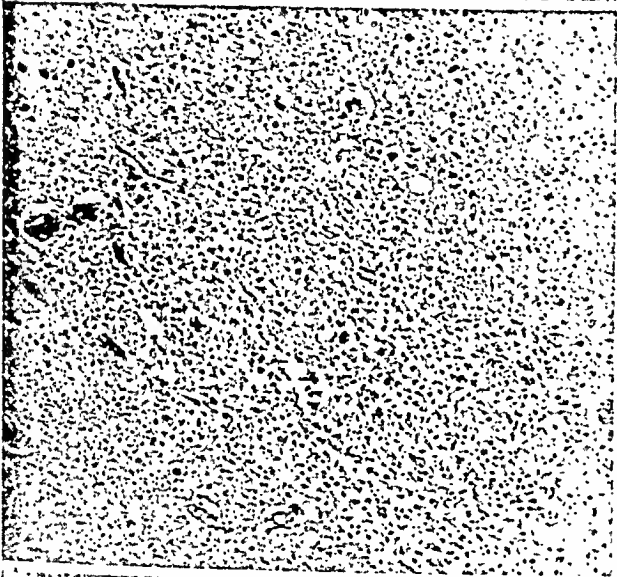
FIG. 15. Case 5. Multiple plaques in cerebral cortex.  $\times 100$ .

FIG. 16. Case 5. Plaque in pontile nucleus containing a few small round cells.  $\times 100$ .

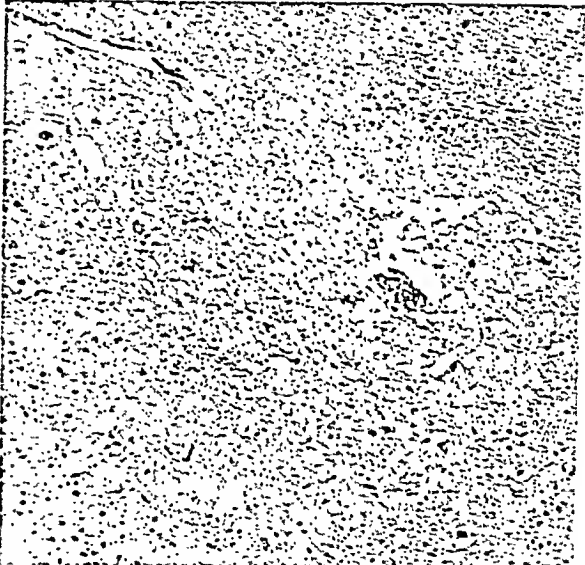
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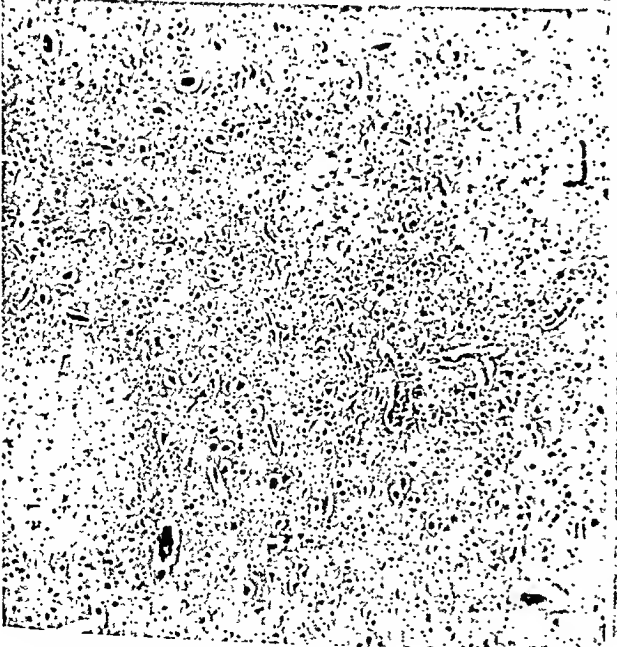
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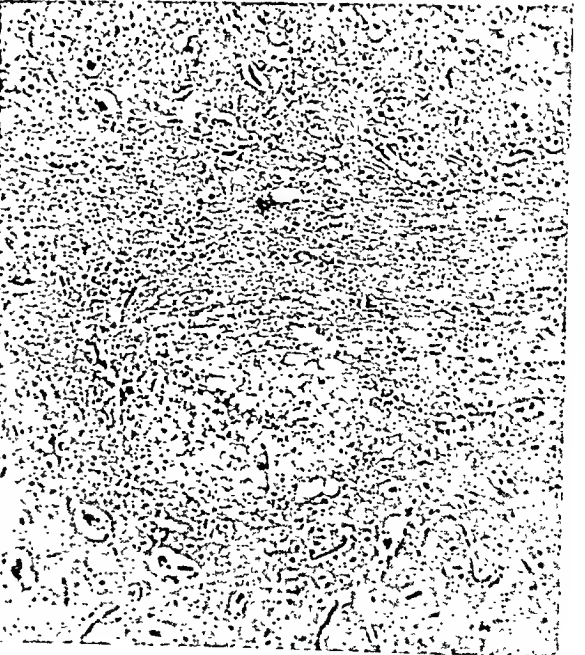


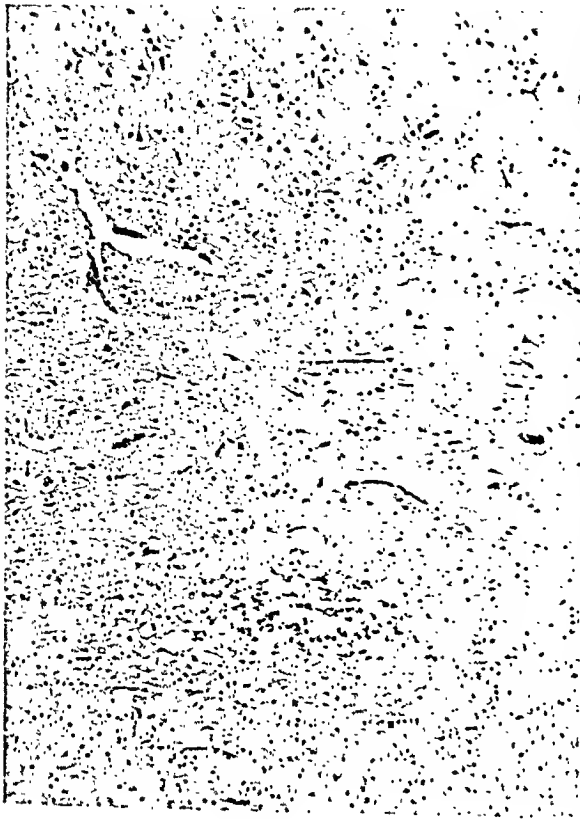
PLATE 185

FIG. 17. Case 5. Focal lesion in sixth layer of cerebral cortex. No encroachment on subcortical white matter.  $\times 100$ .

FIG. 18. Case 5. Fragmentation of axones and dendrites in cortical plaque. Trelles' stain.  $\times 100$ .

FIG. 19. Case 6. Lesions in pontile nuclei encroaching on intervening fiber tracts. Ganglion cells in various stages of disintegration.  $\times 100$ .

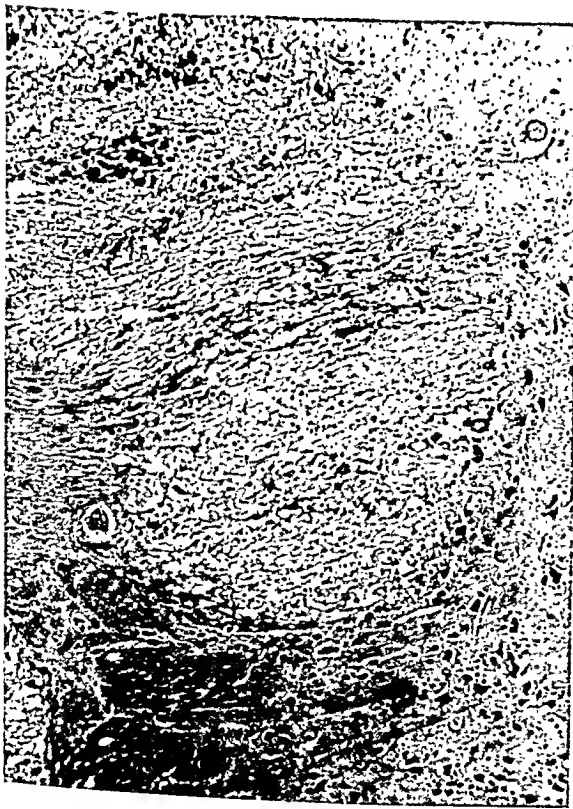
FIG. 20. Case 6. Lesion in pons sharply limited to nuclear mass. Adjacent white matter intact.  $\times 100$ .



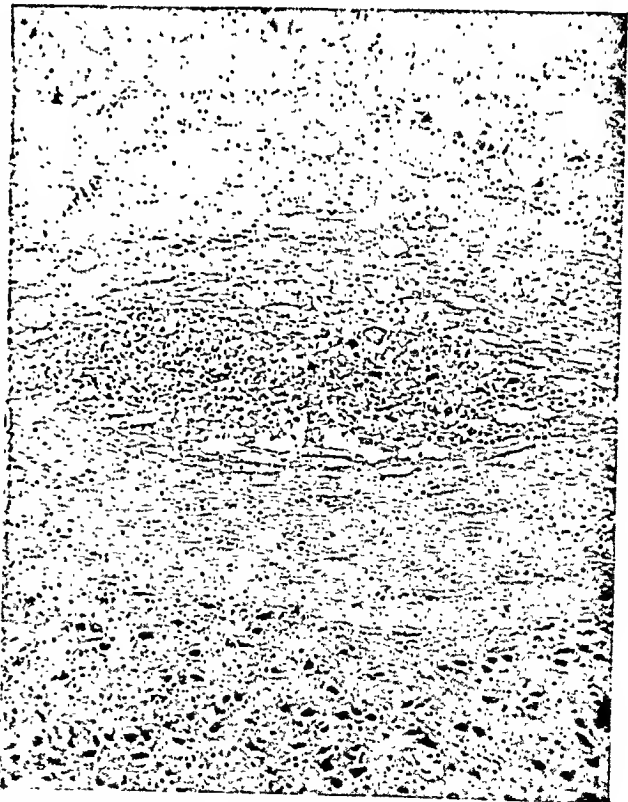
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PLATE 186

FIG. 21. Case 11. Sites of old injuries in pontile nuclei. There is contraction of the original lesions by scar tissue of gliogenous origin.  $\times 100$ .

FIG. 22. Case 7. Glial "lawns" in molecular layer of cerebellar cortex at sites of injured Purkinje cells.  $\times 100$ .

FIG. 23. Case 2. Purkinje cells absent and their former positions indicated by proliferation of glia in the molecular layer of cerebellar folium.  $\times 100$ .

FIG. 24. Case 5. "Spongy" lesion in cerebellar cortex showing fragmentation and disappearance of dendritic processes. Trelles' stain.  $\times 100$ .

FIG. 25. Case 11. Deposit of particles of calcium salts in thalamus.  $\times 100$ .

FIG. 26. Case 11. Diffuse deposition of fine, black particles of calcium salts in cerebral cortex. Von Kossa's stain.  $\times 100$ .

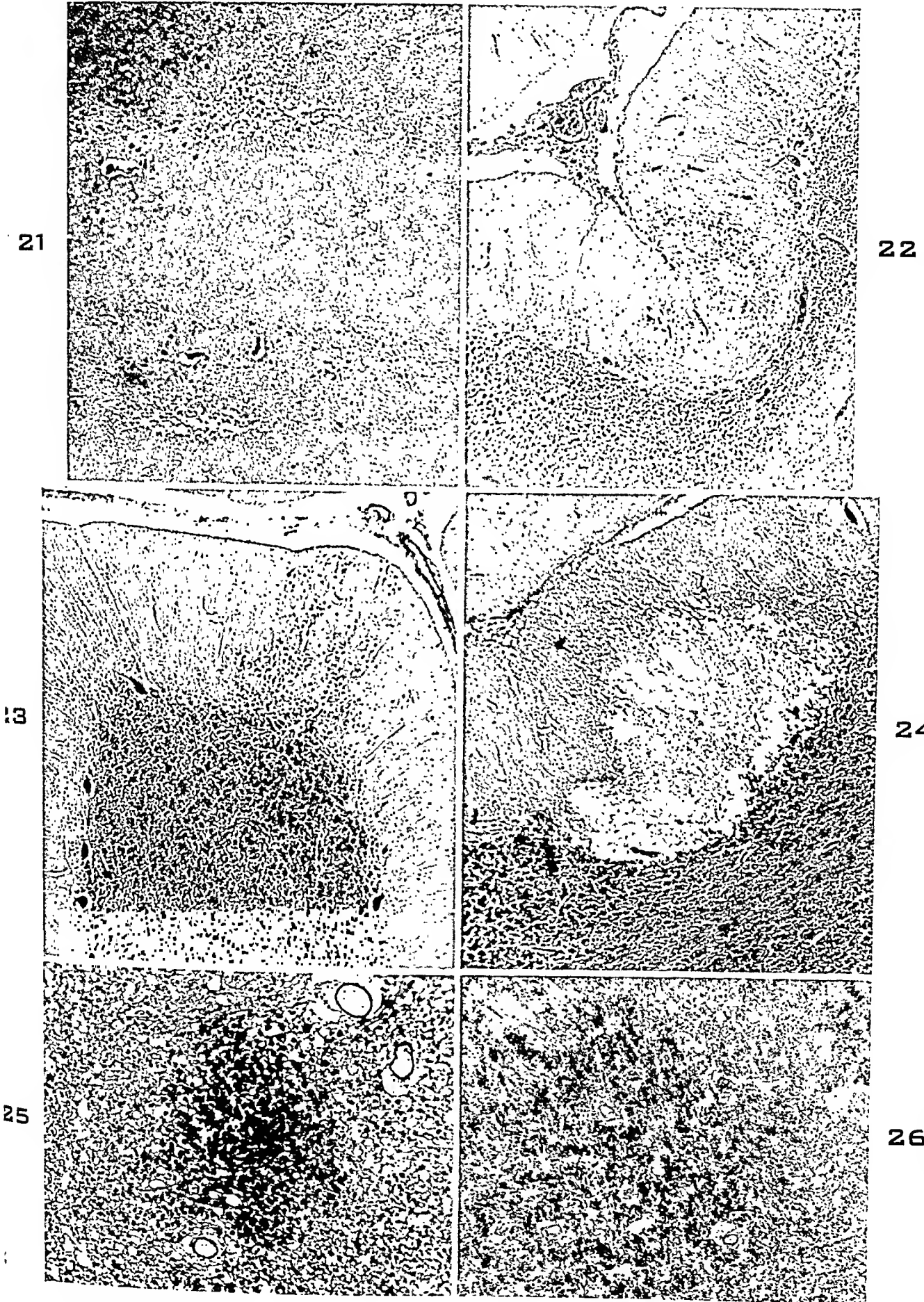


PLATE 187

FIG. 27. Case 5. Multiple plaques of degeneration affecting Purkinje cells and molecular layer.  $\times 100$ .

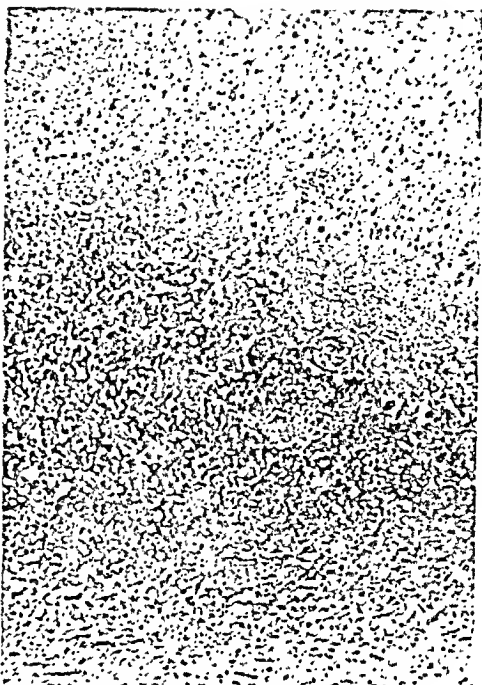
FIG. 28. Case 11. Replacement of all the cortical laminae in the hippocampal gyrus by particles of calcium salts.  $\times 100$ .

FIG. 29. Case 11. Patches of encephalomalacia with deposition of calcium salts in cerebral cortex.  $\times 100$ .

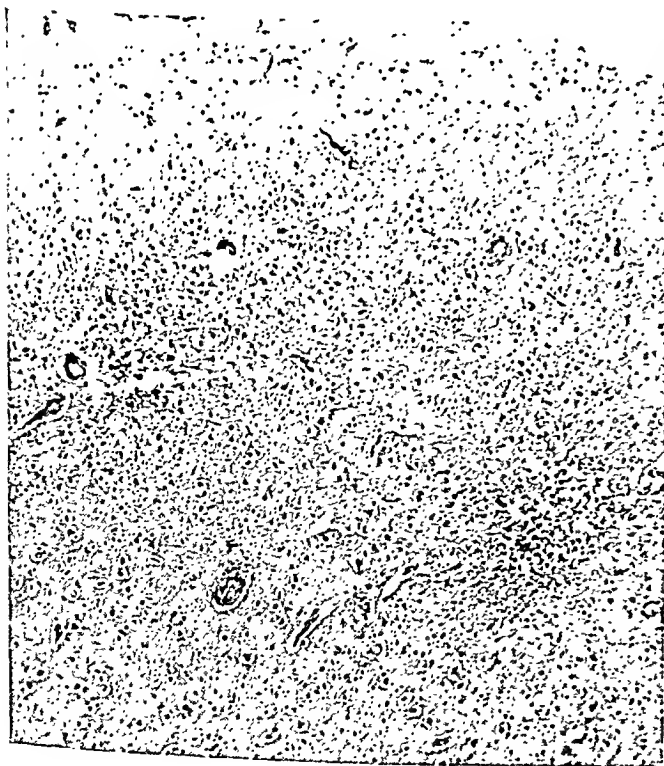
FIG. 30. Case 11. Cystic degeneration in substantia nigra. Black particles are calcium salts.  $\times 100$ .



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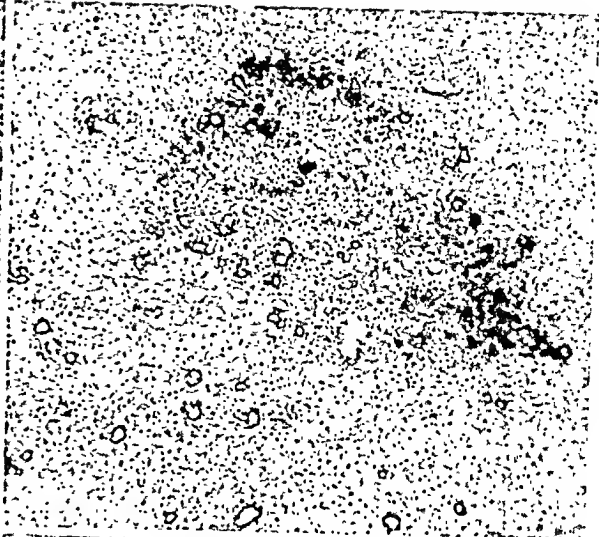
PLATE 188

- FIG. 31. Case 10. Deposit of calcium salts on periphery of patch of encephalomalacia in globus pallidum. The black rings are calcified blood vessels.  $\times 85$ .
- FIG. 32. Case 10. Large accumulations of calcium salts in thalamus.  $\times 85$ .
- FIG. 33. Case 10. Coarse, black granules of calcium salts shown by von Kossa's stain. Region of brain adjacent to that illustrated in Figure 32.  $\times 85$ .
- FIG. 34. Case 10. Multinucleated giant cell has engulfed several particles of calcium salts. Of note are the adjacent large, foamy macrophages. Globus pallidum.  $\times 295$ .
- FIG. 35. Case 10. Multinucleated giant cells, mononuclear phagocytes, and calcium salts in thalamus.  $\times 295$ .
- FIG. 36. Case 10. Complete loss of Purkinje cells and reduction in number of granular cells in cerebellar folium. Bergmann glia unaffected.  $\times 85$ .

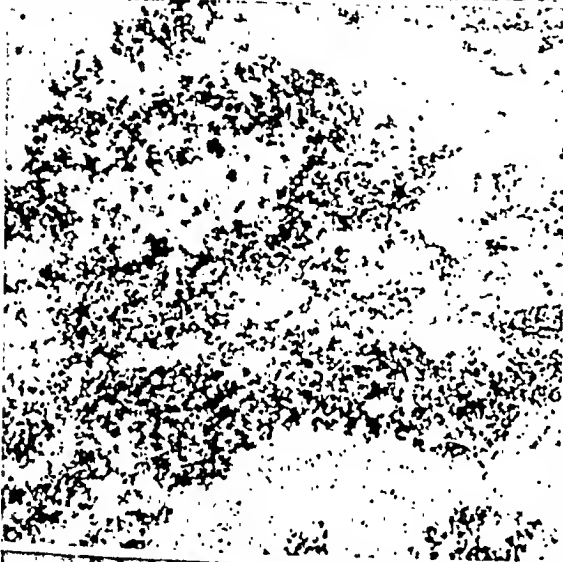
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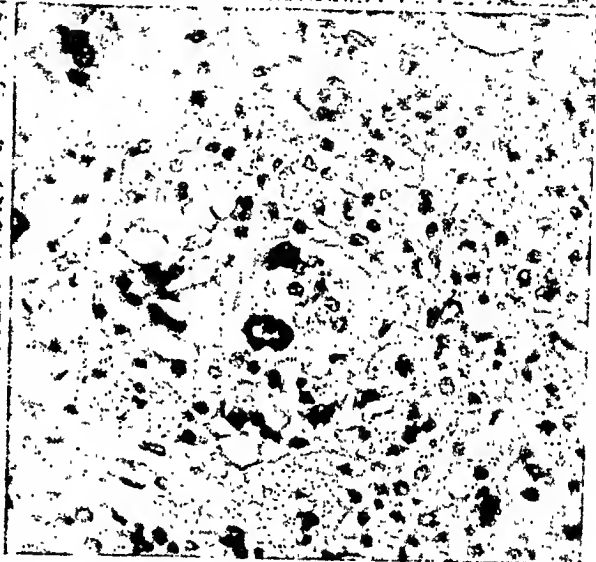
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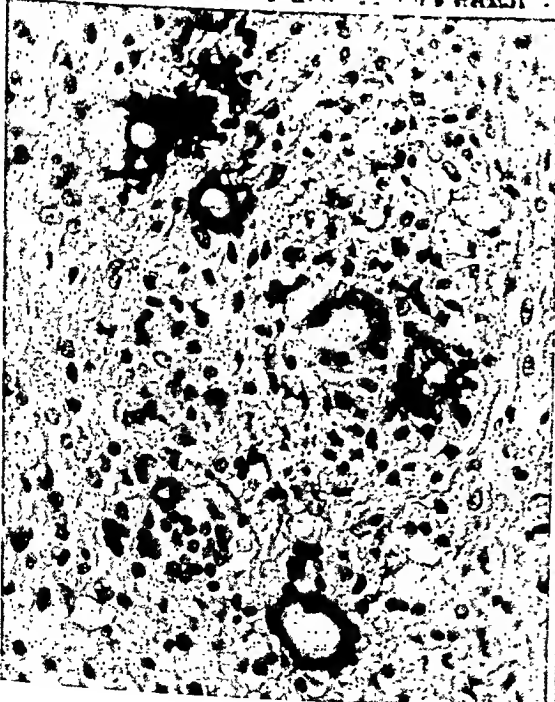
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# HUMAN SALMONELLOSIS DUE TO SALMONELLA SENFTENBERG \*

THEODORE J. CURPHEY, M.D.

(From the Pathological Laboratories, Meadowbrook Hospital, Hempstead, N.Y.)

The recent rapid advances in the field of intestinal bacteriology, more especially as they concern the organisms of the Salmonella group, prompt the reporting of a case of intestinal infection with autopsy findings in which a rare strain, namely *Salmonella senftenberg*, was isolated.

## REPORT OF CASE

**History.** L. G., a white male, 62 years of age, a waiter in a nearby country club, was admitted to Meadowbrook Hospital on December 30, 1944, and died on March 8, 1945. His chief complaint was diarrhea of 2 weeks' duration. He had been well previously, except for occasional attacks of indigestion. At the time of onset he developed severe and persistent vomiting and diarrhea, having as many as 50 watery, foul-smelling stools daily. During this time there had been marked weight loss. There was no history of jaundice, hematemesis, or melena.

**Physical Examination.** Physical examination showed a dehydrated, elderly white male. His tongue was very red. The lungs showed slight impairment of resonance on the right side posteriorly in the region of the third and fourth ribs. The breath sounds were generally diminished over both lungs. The heart was normal. Examination of the abdomen failed to reveal tenderness or organ enlargement. A left scrotal hernia was present. The extremities were normal.

**Laboratory Data.** **Urine:** Negative on two examinations, with presence of an occasional hyaline cast in another sample about 3 weeks prior to death. **Blood:** Four days after admission his hemoglobin was 105 per cent; red cell count, 5,000,000 per cmm.; white cell count, 12,000 per cmm.; neutrophils, 60 per cent; lymphocytes, 40 per cent. Four days later the hemoglobin was 112 per cent. A week later (2 weeks after admission) the hemoglobin was 76 per cent; white cell count, 4,000 per cmm.; neutrophils, 42 per cent; lymphocytes, 58 per cent. One month later the hemoglobin was 72 per cent; leukocytes, 5,000 per cmm.; neutrophils, 55 per cent; lymphocytes, 45 per cent. **Chemical Examination of the Blood:** On admission, nonprotein nitrogen, 72 mg. per 100 cc.; sugar, 130 mg. per 100 cc. Two weeks later, nonprotein nitrogen, 25 mg. per 100 cc.; creatinine, 1.5 mg. per 100 cc.; sugar, 85 mg. per 100 cc. Total serum proteins on this date were 3.3 per cent with 1.4 per cent albumin and 1.9 per cent globulin. The total cholesterol was 160 mg. per 100 cc. Total protein 1 month after the first determination and 6 weeks from the date of admission showed a total of 4.6 per cent with 2.5 per cent albumin and 2.1 per cent globulin. Five days before death the acid phosphatase determination was 3.7 King-Armstrong units; the alkaline phosphatase, 17.3 King-Armstrong units. Wassermann and Kahn tests gave negative results. Cephalin flocculation test performed 5 days before death showed a 1 plus reaction. Serum taken for Widal test on day of admission agglutinated *Bacillus typhosus* (alcoholic antigen) in a dilution of 1:80. When the test was repeated 1 month later, no agglutination with typhoid antigen was obtained. **Gastric Contents:** An analysis done 12 days after admission showed no free acid, no blood, total acid of 4° in the fasting specimen and 12° in the first hour specimen. The material was insufficient for further acid determination in the second, third, and fourth hour samples. **X-Ray Study:** A roentgenogram of the gastrointestinal tract, taken 2 weeks after admission, showed considerable dilatation of the small intestine by gas but no evidence of delay of the progress of the meal. The roentgenologist

\* Received for publication, September 7, 1945.



interpreted these findings as evidence of spasticity of the small intestine. The barium meal showed also marked colonic stasis and a loop of large intestine in the scrotal sac.

*Bacteriological Identification.* A sample of feces obtained 6 days after admission was forwarded to the Salmonella Center of the New York State Department of Health Laboratories, at which time a preliminary report was given on the isolation of an organism of the Salmonella group. A subsequent final report stated that this had been determined to be *S. senftenberg*.\*

*Clinical Course.* On admission the patient's temperature was 97° F. Subnormal temperature persisted for the next day, but on the third hospital day the temperature reached 99° F. For the next 3 weeks it ranged for the most part in the subnormal zone; during the fourth and fifth week the temperature was normal; during the sixth and seventh week and part of the eighth week it was again subnormal, ranging between 97° and 98° F. The pulse rate varied between 70 and 80. Respiration rate ranged between 18 and 25 during the first week and between 15 and 20 subsequently. About 1 month after admission the patient had edema of both ankles and ascites. He was placed on a high protein diet, following which the serum proteins were determined and showed an appreciable increase over the previous test. An electrocardiogram taken 1 month after admission showed low voltage in all three leads. During a good part of the clinical course it was necessary to resort to tube feeding. The diarrhea appeared to be controlled, but recurred when refusal of food led to tube feeding. The stools were green, and during the first week of admission varied from one to two daily and continued so for the second and third weeks. On the fourth week they increased in frequency to three and four daily. On the fifth week the bowels moved once daily. During the sixth week the bowel movements averaged two to four daily. In the seventh and eighth weeks he had two stools daily. In the ninth week diarrhea was noted. During the last few weeks of his life the patient was apathetic, listless, and showed marked anorexia with increasing weakness. During the last 2 days of his life he had large dark brown stools, one or two daily. Immediately prior to death multiple purpuric areas were noted on the dorsa of the hands. He died on the 68th hospital day. Autopsy was performed 5 days later, the body having been unclaimed.

#### AUTOPSY FINDINGS

The body was markedly emaciated and poorly developed, measuring 5 ft. 10 in. in length and weighing approximately 160 lbs. The lips were dry and the tongue parched. No external evidence of lymphadenopathy was seen. There was marked atrophy of the subcutaneous fat. The abdomen was scaphoid. There was rather extensive scaling and hyperkeratosis of the skin, particularly over the legs. On opening the peritoneal cavity the structures were dry in appearance. The heart weighed 200 gm. The myocardium was unusually flabby and on section revealed a light grayish red surface. The coronary vessels were normal. The aorta showed minimal arteriosclerotic change. The lungs showed no noteworthy changes. The liver weighed 860 gm. The capsule was light brown and smooth, the lower edge sharp. The cut surface showed dark brown tissue in which the usual lobular markings were normal. The gallbladder was markedly contracted and contained

\* I wish to express my appreciation to Dr. Ruth Gilbert and Miss Marion Coleman of the Division of Laboratories and Research of the New York State Department of Health for their valuable assistance in the identification of this organism.

several irregular calculi, the wall being thick and fibrotic. The spleen weighed 130 gm. The capsule was grayish red and smooth. On section the cut surface showed a soft, light grayish red pulp. The follicular markings were not visible. The knife scrapings were greatly increased. The esophagus was normal. The stomach was greatly distended with approximately 500 cc. of semi-solid food material and a large amount of gas. The rugal folds were greatly flattened. The mucosal surface was reddish brown. No ulcers were seen. Within the small intestine, particularly in the mid-jejunal area, numerous widely separated, minute, superficial, blackish red ulcers were found, measuring from 1 to 3 mm. in length. They were oval and appeared to be situated on the crests of the valvulae conniventes. The tissue was moderately hyperemic around many of the ulcers. A few ulcers were found also in the lower ileum. None was present in the proximal jejunum. The Peyer's patches were prominent but showed no evidence of ulceration. The ascending colon and sigmoid showed large, irregular, brownish black, shaggy ulcers with a pseudomembranous covering and measuring up to 7 cm. in diameter. In addition, there were several small, moderately deep, oval ulcers measuring 1 cm. in diameter. These grossly resembled those in the small intestine but were deeper and many of them presented sharply sloping edges. The uninvolved mucosa of the large gut showed a deep blackish blue discoloration. The regional mesenteric lymph nodes as well as the peripancreatic nodes were moderately and discretely enlarged, measuring up to 2 cm. in greatest diameter. On section they cut with less resistance than normal to reveal a pinkish red, homogeneous, edematous-appearing surface. The pancreas and adrenals showed no noteworthy changes. The right and left kidneys weighed 120 gm. each and showed no noteworthy gross change. The prostate was moderately enlarged and nodular. The left internal inguinal ring showed a large hernial opening through which the fingers could be passed into the scrotum. No bowel or omentum was present in this sac. Sections of bone showed normal appearing dark red marrow.

*Anatomical Diagnoses.* Acute and subacute ulcerative and pseudomembranous enteritis. Acute mesenteric and pancreatic lymphadenitis. Acute myocardial degeneration. Chronic cholecystitis with lithiasis. Acute splenic tumor. Hyperkeratosis of abdomen and extremities. Dehydration, avitaminosis (?).

#### *Microscopical Findings*

The pertinent histological changes were seen in the gastrointestinal tract and the regional lymph nodes. A typical lesion in the small intestine (Fig. 1) showed the shallow character of the ulceration of the

mucosa, the majority of the ulcers lying superficial to the muscularis mucosae. The mucosal and submucosal areas contiguous to the zone of ulceration were heavily infiltrated with mononuclear leukocytes (Fig. 2), many of the submucosal lymphatics being dilated and filled with these cells.

The lymph nodes from the mesenteric and peripancreatic areas (Figs. 3 and 4) showed interstitial edema and crowding of the sinusoids with many mononuclear leukocytes. The pericapsular fat was heavily infiltrated with mononuclear cells and the lymphatics immediately adjacent to the nodes were markedly dilated and packed with lymphocytes and mononuclear leukocytes (Fig. 5). At a higher magnification (Fig. 6) many of the mononuclear leukocytes showed macrophagic activity in which the cytoplasm of the cell was seen to contain particles of pyknotic-appearing basophilic debris. These cells were identical in morphological and tinctorial qualities with the macrophages seen in typhoid fever, and, indeed, many of the histological features observed in the ulcers of the intestine and in the lymph nodes were quite similar, if not identical, to those seen in that disease.

### *Bacteriological Study*

The feces, as well as a representative ulcer of the intestinal tract, were saved for culture. From each of these *S. senftenberg* was isolated in the State Department of Health Laboratories, as well as in our own laboratory.

The organism was a gram-negative, nonmotile bacillus producing acid and gas in dextrose, maltose, and mannitol. It failed to ferment lactose or sucrose and did not produce indole. In Kligler's medium it produced acid with gas in the butt. The medium was blackened due to hydrogen sulfide production. Further cultural and serological study at the State Department of Health Laboratories established that it was a strain of *S. senftenberg*.

### DISCUSSION

With the pathological findings at autopsy mainly localized in the gastrointestinal tract, and the recovery before death of a species of *Salmonella* from the feces, it would seem probable that the organism was the cause of the ulcerative changes found in the intestinal tract. The clinical history of gastrointestinal symptoms with diarrhea would lend further credence to this belief and would thus make it reasonable to assume that this was a fatal case of relapsing subacute ulcerative enterocolitis caused in all probability by *S. senftenberg*. If these facts are acceptable, then this is the first reported fatal case in a human being caused by this species of *Salmonella*.

This organism has been encountered previously in veterinary and medical material. Edwards,<sup>1</sup> in 1937, isolated it in connection with an epidemic in turkeys. He called attention to the fact that the serological reactions established a similarity between it and the Jena and Dublin varieties of *S. enteritidis*. He found that the organism produced acid and gas in glucose, trehalose, arabinose, xylose, sorbitol, and dulcitol, but that lactose, sucrose, and inositol were not attacked. He further noted that hydrogen sulfide was formed and that prompt production of acid developed in tartrate agar. Edwards' cultures were found to be serologically identical with the Senftenberg type of Kauffmann<sup>2</sup> (1929) and the Newcastle type of Warren and Scott.<sup>3</sup> He stated that Kauffmann and Mitsui,<sup>4</sup> in 1930, demonstrated that the only difference in these types is the production of hydrogen sulfide by the Senftenberg strain and the failure of the Newcastle strain to produce it. It must be noted, however, that in Warren and Scott's report in 1929 they stated that a new serological type of *Salmonella* isolated by them and designated as Newcastle I and II did show very feeble production of hydrogen sulfide with slow and slight blackening of lead acetate medium. Edwards' strains were isolated from young turkey poults, showing icteric livers and joint involvement, with a low mortality rate (not exceeding 10 per cent) in the flock examined. It is of note that this report of Edwards is the first account in which *S. senftenberg* was definitely associated with a pathological process in man or animals.

In dealing with *Salmonella* infections in man, a recent report by Seligmann, Saphra, and Wassermann<sup>5</sup> details an analysis of 1,000 cases bacteriologically identified by the New York *Salmonella* Center. Of these 1,000 cases, *S. senftenberg* was isolated from only 9. The organism was recovered from the stool in each case and was obtained in five outbreaks of disease, all coming from a limited episode in an asylum in Massachusetts. Furthermore, of these 9 cases, 4 patients were healthy carriers, the other 5 showing symptoms of gastro-enteric disease. In none of these 5 cases was there a fatal outcome. In 1942, Bornstein and Saphra,<sup>6</sup> in a report of certain unusual *Salmonella* types isolated by them, stated that this organism (*S. senftenberg*) had previously been found by them in Chinese eggs and had also been isolated from a human carrier. In 1943, Edwards and Bruner<sup>7</sup> examined 3,090 cultures of *Salmonella* isolated from 2,285 outbreaks of infection in man and animals. *S. senftenberg* was isolated from 15 outbreaks in fowls, 4 outbreaks in swine, and 4 outbreaks in man. Furthermore, of the 10 cultures of *S. senftenberg* isolated in man during this study, 8 were recovered from normal carriers and 2 from cases showing symptoms of gastro-enteric disease. Neither of these 2 cases was fatal. From the above evidence it can be seen that the organism is a rare

invader when compared to the other *Salmonella* types. It shows, too, that it is often isolated from human carriers showing no evidence of disease.

This ability to recover *Salmonella* from human carriers makes it necessary to exercise caution in imputing a causal relationship between any pathological process found at autopsy and a *Salmonella* strain recovered either before death or from autopsy material. Thus, Seligmann, Saphra, and Wassermann<sup>5</sup> stated: "The finding of *Salmonella* in a fatal case does not of necessity imply that the recovered organism is the sole or even a contributory cause of death." It appears, however, from a study of the clinical course in my case, coupled with the autopsy findings, that no other demonstrable cause of death was determined and that the main pathological changes were found in the gastrointestinal tract. The recovery of the organism from the stool during life and at autopsy from the fecal contents and intestinal ulcers would seem to warrant the conclusion that the organism was pathogenic for this host and was the cause of the ulcerative lesions. While too much significance cannot be attached to the fact that the mucosa of the intestinal tract showed marked hydrogen sulfide staining because of the length of time between death and autopsy, nevertheless the knowledge that *S. senftenberg* is a hydrogen-sulfide-producing organism might be adduced as confirmatory evidence that the organism can be incriminated as the etiological agent responsible for the ulcerative lesions found in the intestine.

While it is hazardous to attempt to outline the pathogenesis of *S. senftenberg* infection in the human being from the study of a single case, nevertheless the clinical history and the gross and microscopical findings in this case seem sufficiently similar to those of certain well established enteric diseases to warrant the attempt. Thus it is obvious from the gross and microscopical studies that this infection is essentially like that seen in paratyphoid fever caused by *S. paratyphi* and *S. schottmülleri*, respectively. For instance, the gross distribution of the ulcerative change in the small and large intestine, with pseudomembranous inflammation, is reminiscent of paratyphoid fever in contrast to the greater tendency to localization of ulceration in the small intestine in typhoid fever. In addition to the distribution of the lesions is the fact also that grossly and microscopically, *S. senftenberg* infection is quite like that of paratyphoid fever in that the ulcers are superficial, being confined for the most part to the mucosal layer and the upper layers of the submucosa, with the characteristic mononuclear cellular exudate seen in these typhoid-like infections of the gastrointestinal tract. The dense mononuclear cellular infiltration of the

lymphatics and the diffuse mononuclear proliferation with evidence of phagocytosis in the regional lymph node also conform to this picture.

It is of interest to consider the relationship between the clinically observed protracted gastro-enteric disease with periods of acute exacerbation and remission and the pathological findings. Thus the gross inspection failed to reveal areas in the small or large intestine that could be interpreted as evidence of healed ulceration as is sometimes seen in infections with closely allied organisms, *e.g.*, *Bacillus typhosus*. If the ulcers seen at autopsy were typical of those present during the previous exacerbations as suggested by the periodic attacks of diarrhea, their very superficial nature would suggest that complete resolution of the mucosa occurs during the period of remission, only to be followed by fresh areas of ulceration during a relapse in the disease. There is thus no controvertible evidence in the material here submitted that *S. senftenberg* in the human being does not behave exactly like other known Salmonella infections. Moreover, this strong resemblance might be adduced as sound evidence that the organism isolated from the feces and ulcers during life or at autopsy was in all probability the true inciting agent of the pathological changes present in this case.

Another point of general interest in this case is the fact that the patient was a food-handler who could well have harbored the organism for many years prior to the actual symptoms of his disease. *S. senftenberg* is more often found in normal human carriers than as a disease incitant and sporadic outbreaks of Salmonella infections are frequently due to food-handlers who are carriers of the organism.

In conclusion, it would seem that the importance of the correct identification of intestinal incitants of disease has not received sufficient emphasis. For example, it is the general practice to terminate the routine bacteriological investigation of submitted fecal material after the usual cultural and serological approaches have been made towards the identification of the common incitants found in the typhoid-colon-dysentery group. Frequently an organism having cultural features that do not readily place it in this large group is not studied in greater detail because its possible significance in the causation of the intestinal disease is under-estimated. It is this type of organism that should receive more extended study in the hands of an experienced laboratory group working with strains of Salmonella. The very multiplicity of the Salmonella types would tend to emphasize the need for accurate identification by special technical approaches, if the epidemiology, epidemiology, and control of salmonellosis are to be the subjects of serious study. Furthermore, the experience in this single case would lead one to suspect that many of the types of Salmonella that

are now established as not being pathogenic for man might be found to be disease incitants if more detailed cultural studies were made on clinical and autopsy specimens by those specially skilled in isolating and identifying these organisms. With this in mind, it would seem desirable to study by cultural methods all cases which at autopsy present inflammatory gastrointestinal lesions. Whenever an organism is recovered which cannot be readily identified, it should be submitted to a *Salmonella* center for further study.

### SUMMARY

1. What is believed to be the first reported example of fatal human intestinal infection by *Salmonella senftenberg* is described. The organism was isolated during life and at autopsy from a patient with recurrent diarrhea.

2. Emphasis is placed on the importance of more detailed investigation of organisms of the enteric group having atypical cultural and serological features when such are recovered during life or at autopsy from patients showing gastro-enteric symptomatology or lesions.

3. Any organism recovered during life or at autopsy from cases of enteric disease and not readily identifiable by ordinary laboratory procedures should be forwarded to one of the *Salmonella* centers for investigation and identification.

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[ *Illustrations follow* ]



## DESCRIPTION OF PLATES

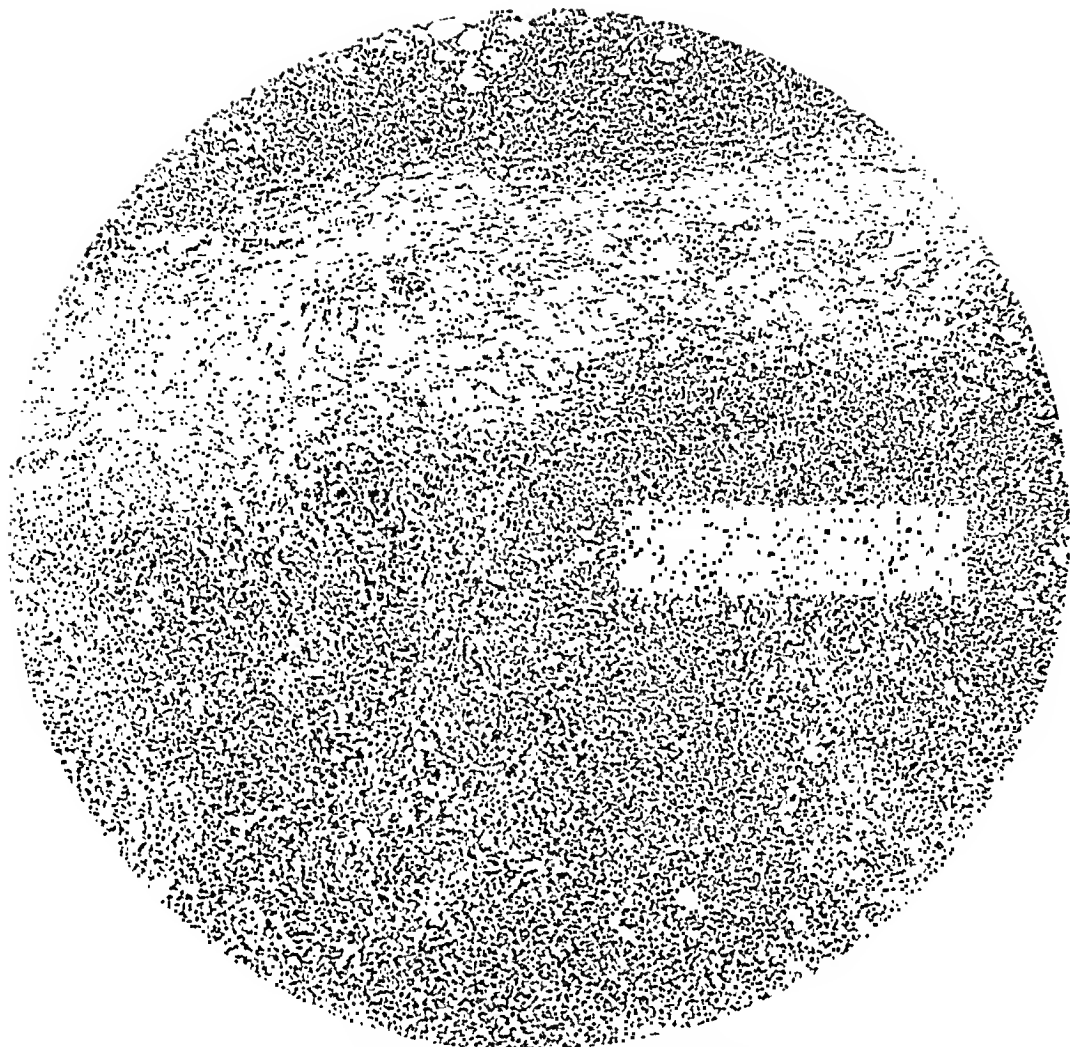
### PLATE 189

FIG. 1. Section of an ulcer of the small intestine showing its superficial location above the level of the muscularis mucosae. The pattern of the adjacent mucosal gland is lost due to marked post-mortem autolysis. Hematoxylin and eosin stain.  $\times 8$ .

FIG. 2. Higher magnification of Figure 1 to show the mononuclear cellular infiltration of areas contiguous to the zone of mucosal ulceration. A dilated submucosal lymphatic filled with these cells is seen in the center of the field. Hematoxylin and eosin stain.  $\times 20$ .

FIG. 3. Lymph node densely packed with mononuclear cells with associated fibrous thickening of the capsule and dense pericapsular mononuclear cellular infiltration. Hematoxylin and eosin stain.  $\times 15$ .

3



Curphey

2



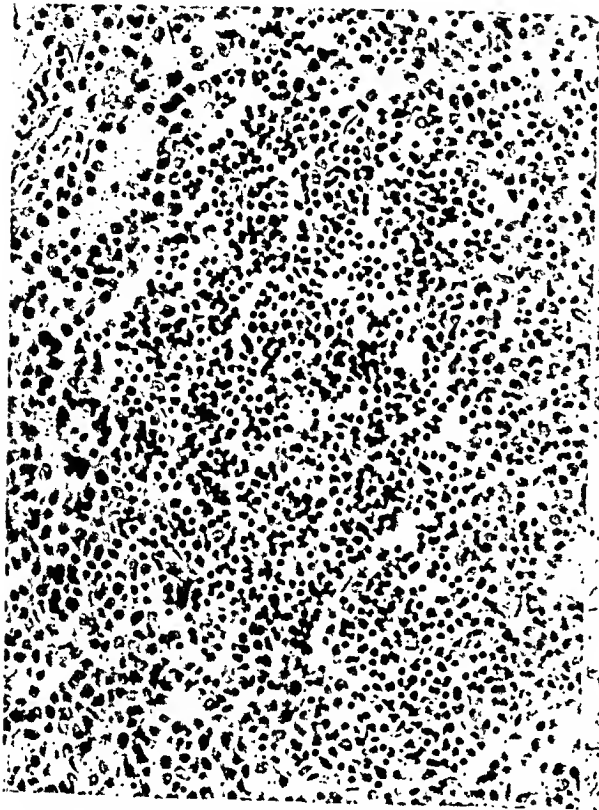
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PLATE 190

- FIG. 4. Higher magnification of Figure 3 showing a well preserved lymph follicle surrounded by an edematous reticulum rich in lymphocytes and mononuclear leukocytes. Hematoxylin and eosin stain.  $\times 180$ .
- FIG. 5. Perinodal fat containing dilated lymphatics packed with lymphocytes and mononuclear leukocytes.  $\times 25$ .
- FIG. 6. Higher magnification of Figure 4 showing in the upper left-hand portion of the field the presence of typical macrophages with phagocytized debris. Hematoxylin and eosin stain.  $\times 400$ .

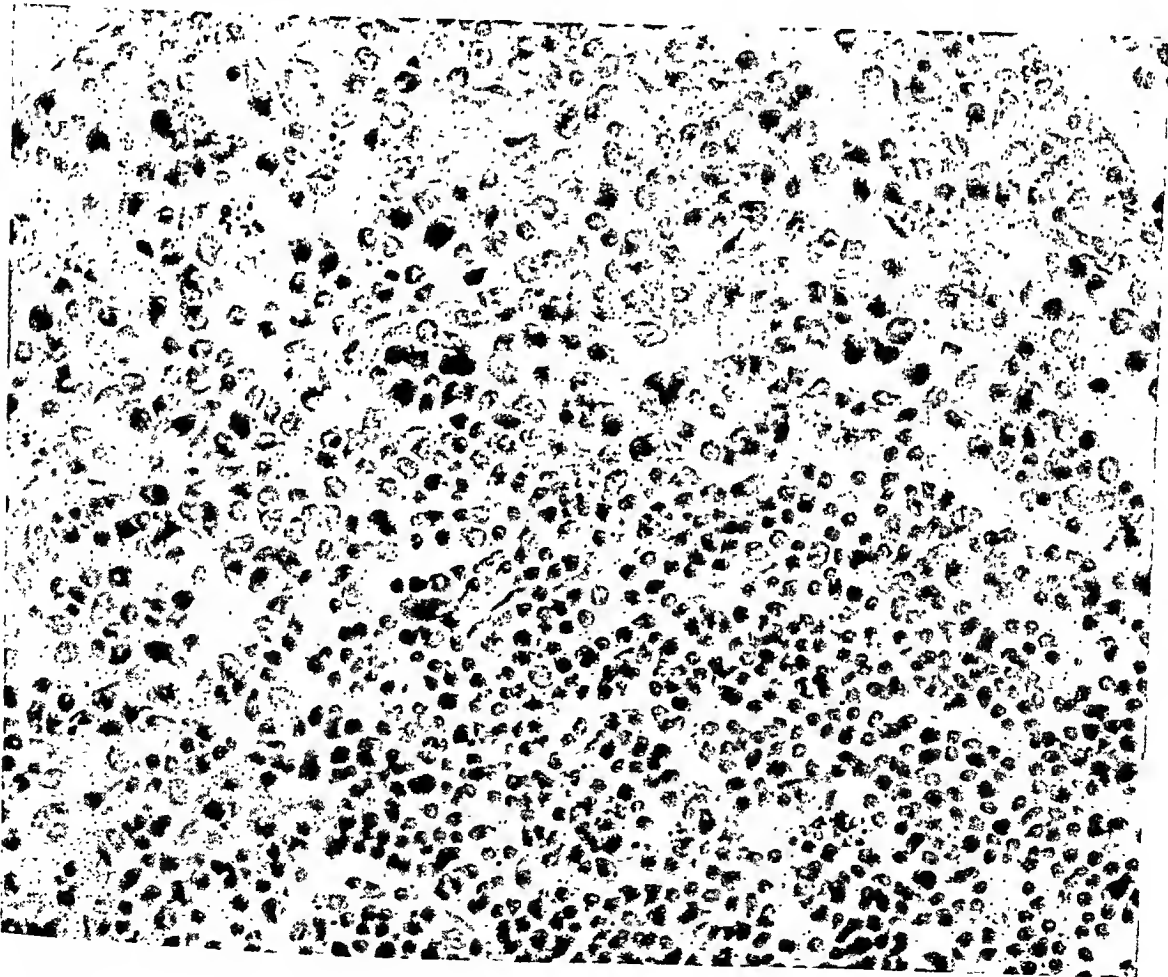
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Curphey



# ARTERIAL CALCIFICATION IN INFANCY WITH SPECIAL REFERENCE TO THE CORONARY ARTERIES \*

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Unlike occlusive lesions of the coronary arteries of older children and adults, which may result from any one of several diseases,<sup>1</sup> occlusion of these arteries in infants is nearly always due to a single type of arterial disease. This lesion consists of calcification of the wall of the artery with fibroblastic proliferation of the intima. The intimal proliferation may be so marked as eventually to cause complete or nearly complete obliteration of the lumen. This lesion may occasionally be found in the arteries of older persons but its comparative significance in that age group is lessened by the more frequent occurrence of arteriosclerosis of the atheromatous type, inflammatory processes, and other arterial diseases.

Fifteen cases of calcification of the coronary arterial wall with intimal proliferation in infants have been previously reported. These cases are collected in Table I. In some instances this involvement of the coronary arteries has been the cause of death; in others, similar involvement of other vessels has been fatal. The ages of the 15 patients ranged from 1 day to 27 months. Seven were male and 8 female. In all cases, other arteries were involved in addition to the coronary arteries. Five children died suddenly, while others had presented varying clinical evidences of cardiac failure.

In the Department of Pathology of the University of Michigan the hearts of 4 infants with calcification of the coronary arteries have been examined. A fifth case was seen in the pathological service of the Providence Hospital, Detroit. A description of that case is included by the courtesy of Dr. Donald H. Kaump. In one case the lesion was severe enough to be the probable cause of death, while in 3 cases it was a possible contributing cause. In the other case only earlier stages of the process were shown.

## REPORT OF CASES

### Case 1

A male infant, 3 months old (7220-LAI), had been considered ill for only 3 days. Clinical symptoms were described as those of an "influenzal pneumonia." One male sibling was said to have died with similar symptoms. No history was obtained of unusual dietary or other features for either the child or its mother.

Autopsy was performed at another hospital and only portions of

\* Received for publication, October 2, 1945.

TABLE  
Summary of Reported Cases of

Case no.	Author	Year	Age	Sex	Renal lesions	Other lesions	Parathyroids
1	Bryant and White <sup>2</sup>	1891	6 mos.	M	Hydronephrosis		
2	Surbek <sup>3</sup>	1917	3 days	F	Chronic nephritis	Pericarditis; splenomegaly	
3	Verocay <sup>4</sup>	1920	5¾ mos.	F	Focal calcification in renal glomeruli	Congenital lues(?) (no spirochetes demonstrable)	
4	Hughes and Perry <sup>5</sup>	1929	7 wks.	F	No gross abnormality		
5	Förster <sup>6</sup>	1930	3 mos.	M	Low-grade chronic glomerulonephritis	Navel infection; infectious splenic tumor	
6	Iff <sup>7</sup>	1931	1 day	M	Glomerular and intertubular calcification		Normal
7	Lightwood <sup>8</sup>	1932	27 mos.	F	Glomeruli fibrotic and calcified; interstitial fibrosis; epithelial calcification	Bronchopneumonia; epiphyseal lines broad, irregular; osteoporosis and osteosclerosis	One identified; normal; no evidence of tumor in neck
8	Oppenheimer <sup>9</sup>	1938	6 mos.	F	Calcification of arteries with early scarring	Bones normal	Small; no microscopic lesion
9	Oppenheimer <sup>9</sup>	1938	4 mos.	F	Calcification of arteries with calcification of glomeruli and tubules	Bones normal	Not examined
10	Brown and Richter <sup>10</sup>	1941	3 mos.	M	Swelling of glomeruli and of epithelium of tubules; calcification of arteries		Not examined
11	van Creveld <sup>11</sup>	1941	6 wks.	F	Early glomerulonephritis		
12	van Creveld <sup>11</sup>	1941	9 wks.	M	Early glomerulonephritis		
13	Baggenstoss and Keith <sup>12</sup>	1941	8 wks.	F	Arteriosclerosis of kidney; increased endothelial cells in glomerular capillaries; some hyaline and calcified glomeruli; "no evidence of severe renal disease"		
14	Andersen and Schlesinger <sup>13</sup>	1942	6½ mos.	M	Bilateral hydronephrosis	Early renal rickets; acute lobular pneumonia	5 times normal size; all 4 glands examined
15	Andersen and Schlesinger <sup>13</sup>	1942	5 mos.	M	Polycystic kidneys	No definite rickets by roentgenogram	One examined was 5 times normal size
16	Stryker	1946	3 mos.	M			Not examined
17	Stryker	1946	2 mos.	F	Calcification of tubular epithelium	Acute purulent bronchitis and bronchopneumonia	Not examined
18	Stryker	1946	6 mos.	M		Early acute lobular pneumonia	Not examined
19	Stryker	1946	Stillborn	M	Polycystic kidneys		Not examined
20	Stryker (Kaump)	1946	7 mos.	F	Calcification of arteries	Occasional foci of acute pneumonitis; focal fibrosis and calcification of myocardium; fibrosis of endocardium of left ventricle	Not examined

organs were available for microscopic examination. Gross findings were not noted. Microscopic examination of the coronary arteries showed heavy calcification of the internal elastic membrane and adjacent inner portion of the media (Fig. 2). Central to this calcification the lumen

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*Coronary Arterial Calcification in Infants*

Vitamin D	Syphilis	Other factors	Congenital abnormalities	Family history
"In physiological quantities only"	Wassermann of mother negative	Sudden death	Congenital urethral dilatation	Mother: developed otitis on third puerperal day; low-grade infection during pregnancy(?) Syphilis
	"Congenital lues"; Wassermann positive (1 plus)	Given antiluetic therapy; clinical rickets	Hydrocephalus(?)	
	Wassermann negative	Sudden death		Mother: had "influenza" during sixth month of gestation
	Wassermann negative			Sibling died suddenly at 6 months
	Wassermann of mother negative	Hydramnion	Abnormal course of aortic arch	Mother: past history of nephritis; two normal older siblings
Given "in normal amount"	Wassermann negative	Albuminuria; high blood pressure; calcium, 11 mg. %; phosphorus, 6.68 mg. %; fever		
		Sudden death		
		Cardiac failure		
None	Kahn of mother negative	Illness of 24 hours' duration		Mother and father: healthy
None	Wassermann of parents negative			Mother: asthmatic; use of lobeline and caffeine during pregnancy; 1 prior abortion
None(?)		Sudden death		Mother: eczema; family history of asthma
				Mother: well during pregnancy
4 times usual dose	Wassermann negative	Calcium, 7.5 to 11 mg. %; phosphorus, 6 to 10 mg. %; nonprotein nitrogen, 55 to 120 mg. %; "otitis media"; death not sudden	Congenital hypoplastic kidneys	7½ months premature sibling died on first day; no familial disease
Given in moderate amounts		Calcium, 6.5 to 9 mg. %; phosphorus, 6.4 to 11.3 mg. %; sudden death		Family history irrelevant
None				Male sibling died with similar symptoms
	Congenital lues; Kahn, 4 plus	"Parotitis"		Syphilis(?)
	No serologic test	"Snuffles"		One miscarriage; sibling died at 5 weeks, cause unknown; one sibling in good health
	Kahn of mother negative		Multiple anomalies	Mother: bronchiectasis; allergic bronchitis
Cod-liver-oil concentrate; 2 drops daily during first month	Serologic test of mother negative	"Chronic cold" for 3 months		"Normal pregnancy"; "family history not contributory"

of one artery was completely occluded by young connective tissue in which there were many newly formed vascular channels. Other main coronary stems showed a partial occlusion of the lumina by a similar process. In this young connective tissue there was an occasional poly-



morphonuclear leukocyte or lymphocyte. No hemosiderinophages were seen. The appearance of the connective tissue was uniform. In the adventitia there was an infiltration of polymorphonuclear leukocytes, lymphocytes, and monocytes; this infiltration was more marked focally but the distribution was not perivascular. Sections stained with van Gieson's stain for connective tissue and with the Verhoeff stain for elastic tissue demonstrated this newly formed tissue to be young fibroblastic connective tissue.

The myocardial fibers were considered to be slightly hypoplastic. In the myocardium were small foci of recent infarction, located chiefly in the subendocardial area and in the papillary muscles (Fig. 1). The finer branches of the coronary arteries appeared normal. Subendocardial vacuolar degeneration was present. Changes in other organs were largely limited to the vessels. The lungs showed acute passive congestion and acute edema. In a large pulmonary artery there were small scattered foci of degeneration and calcification of the wall, and, in smaller branches, focal calcification of the internal elastic membrane. Arteries at the hilum of the spleen and in the pelvis of the kidney (Fig. 3) showed calcification and partial occlusion similar to that in the coronary arteries. The splenic vein contained a recent thrombus and the spleen showed acute passive congestion.

*Pathological Diagnoses.* Marked calcification of the walls of the coronary arteries with associated connective tissue proliferation of the intima; complete occlusion of a main coronary artery; recent myocardial infarcts; calcification and intimal proliferation in splenic, renal, and pulmonary arteries; recent thrombus in splenic vein; acute passive congestion of all viscera; acute pulmonary edema; patchy pulmonary atelectasis and emphysema; cloudy swelling of liver and kidneys.

### Case 2

A female infant, 2 months old (A-268-AJ), was admitted to the hospital because of prematurity. She had been born after 7 months' gestation and at the time of admission weighed only 3 pounds. The delivery had been normal. Slight cyanosis had been noted at birth. She was treated in the hospital as a feeding problem. A routine Kahn test of the blood gave a 4 plus reaction; the result of a second Kahn test was similar. The only family history available was that both parents were living and well; there were no siblings. Antileptic therapy was started. The patient did not gain weight satisfactorily. Additional complications were diarrhea of unexplained cause and a recurrent elevation of the temperature to 102° F. Despite various therapeutic measures she died after 2 months of hospitalization. Clinical diagnoses were prematurity and congenital syphilis.

No lesions were noted on gross examination of the heart. On microscopic examination, however, the coronary arteries showed calcification of their walls of varying extent. In some arteries only a fine basophilic stippling could be seen along portions of the internal elastic lamina,

either on the membrane or closely applied to its margins. In other areas the amount of deposit was greater; one or both sides of the membrane might be affected. The calcification did not involve the entire circumference of the arteries but was present in patchy areas. The internal elastic lamina maintained its identity as an eosinophilic strand through the calcium except for occasional interruptions of its continuity where calcification was most marked. In some of the foci of early deposition of calcium the lamina was basophilic. Over the patchy areas of calcification there was early fibroblastic proliferation of the intima. This did not appear to be sufficient to cause occlusion of the artery but there was diminution of its lumen. No lesions were found in more distal portions of the arteries, nor were lesions seen in the myocardium.

The splenic artery showed a localized area of similar calcification, with calcification also in the adjacent fibroblastic tissue of the intima. The elastic lamina was seen as an eosinophilic band in the calcareous material (Figs. 4 to 6). Fibroblastic proliferation in the intima was not marked. Other significant findings were early purulent bronchitis and bronchopneumonia, and fairly marked calcification of some of the epithelium of the convoluted tubules of the kidneys. A presumptive Kahn test on the heart's blood was positive although the routine Kahn test was negative. There were no histologic evidences of syphilis in any of the tissues.

*Pathological Diagnoses.* Prematurity; early purulent bronchitis and bronchopneumonia; calcification of coronary arteries with early fibroblastic proliferation of the intima and reduction of the lumen without occlusion; localized calcification of the wall of the splenic artery; focal calcification of the renal tubular epithelium; congenital syphilis (?) (positive Kahn reactions).

### Case 3

A colored male infant, 6 months old (A-66-T), was hospitalized because of difficulty in feeding associated with retraction of the head, present since birth. The infant had been born at full term. For the first 7 weeks of life jaundice was said to have been present but this disappeared. The mother had had one miscarriage at 4½ months; a sibling had died at 5 weeks of age of a cause not known, and another sibling, 4 years old, was in good health. The patient had had no acute illness, but "snuffles" had been present for about 8 weeks. During hospitalization the infant frequently refused feedings and became increasingly weaker. He died on the second day after admission. No serologic tests were performed.

At autopsy, all organs showed marked atrophy. Microscopic examination of the heart showed changes in the coronary arteries similar to those described in the previous cases. The deposition of calcium was again along the course of the internal elastic lamina. There were frequent interruptions of the continuity of the lamina. At one level the

fibroblastic proliferation of the connective tissue of the intima was sufficient to decrease the lumen of the vessel (Fig. 7). No myocardial lesions were found.

An artery in periadrenal connective tissue showed a more advanced stage of the lesion (Fig. 8). The internal elastic membrane could not be identified, being included in the areas of calcification, and proliferation of the intima was so great that only a small blood-channel remained. Small giant cells of foreign body type were adjacent to some of the masses of calcium. In the splenic artery there was a localized area of calcification of the wall without intimal change. The internal elastic lamina was included in the area of calcification and showed interruption in continuity.

Other significant findings were chronic passive congestion of the lungs with many hemosiderin-filled macrophages and chronic passive congestion of the liver. Early lobular pneumonia was present. Adipose tissue showed marked atrophy.

*Pathological Diagnoses.* Calcification and fibroblastic intimal proliferation of coronary arteries and of periadrenal and splenic arteries; chronic passive congestion of lungs and liver; early lobular pneumonia; malnutrition with atrophy of all tissues.

#### Case 4

A stillborn male infant (A-179-AL) was delivered after 8 months' gestation. The mother, 14 years old, suffered from chronic pansinusitis, allergic bronchitis, and bronchiectasis. Her Kahn test was negative.

At autopsy, the infant showed multiple developmental anomalies. Sections of the heart showed focal calcification of the wall of one coronary artery near its origin. The internal elastic lamina could be identified at either end of the mass of calcium, but was interrupted in the area of calcification. There were multiple small foci of necrosis and calcification in the myocardium. The lesion in the coronary artery was not occlusive and it is probable that the lesions in the myocardium did not result from it. Focal calcification on both sides of elastic fibers was found also in the pulmonary artery (Fig. 9) and in the first portion of the aorta.

*Pathological Diagnoses.* Stillbirth; multiple disturbances of development: encephalomeningocele, polycystic kidneys, polydactylysm; focal calcification of a coronary artery, the aorta, and the pulmonary artery; focal necrosis of the myocardium.

#### Case 5

A female infant, 7 months old, had been ill for about 3 months with a "chronic cold." The mother had had a normal pregnancy. The infant had been breast-fed; she had received 2 drops of a concentrate of cod-liver oil twice daily during the

first month. There had been no other illnesses. Her condition appeared to be improving, but 2 days before admission to the hospital an elevation of temperature was noted. Two days later her parents noted gurgling sounds in her throat; there was a further rise of temperature and more marked cough. She became cyanotic and was taken to Providence Hospital, Detroit, where she died before any examination could be made. Serologic test of the mother was negative for syphilis. Post-mortem roentgenologic examination showed marked cardiac hypertrophy.

At the autopsy performed by Dr. Donald H. Kaump, the heart weighed 100 gm. The mitral valve appeared slightly thickened, and the endocardium of the left ventricle was pale gray. There was slight, bilateral hydrothorax and gross evidence of purulent lobular pneumonia, confirmed microscopically.

The striking lesions, however, were found in the cardiovascular system. The small arteries and arterioles of the heart, lungs, kidneys, pancreas, and periadrenal adipose tissue showed varying degrees of calcification involving and adjacent to the internal elastic membrane, with proliferation of the connective tissue of the intima (Figs. 10 to 12). A vessel in the peripancreatic tissues was completely occluded except for newly formed vascular channels; a large coronary artery was partly occluded. Study of various vessels indicated that the first change was in relation to the internal elastic membrane, especially on the medial side, with subsequent intimal proliferation and involvement of the entire area previously occupied by the fibers of the media. That the deposited material was lime salts was indicated by the use of dilute hydrochloride acid on sections. Some of the muscle fibers of the media showed hyaline change. In some vessels a foreign body giant cell reaction to the calcareous masses was seen. The endocardium was thickened by proliferation of fibrous tissue. In the myocardium were scattered foci of fibrosis and in some of these calcification had occurred. Remaining myocardial fibers showed hypertrophy. Renal changes were limited to lesions in the arteries and arterioles.

*Pathological Diagnoses.* Calcification of the wall and fibroblastic proliferation of the intima of coronary, pulmonary, renal, pancreatic, and periadrenal arteries and arterioles; cardiac hypertrophy; fibrosis of the endocardium of the left ventricle; focal myocardial fibrosis with calcification; bilateral pulmonary edema; bilateral hydrothorax; bilateral acute purulent lobular pneumonia.

#### DISCUSSION

The essential pathologic lesion in each of these cases is the same; the varied histologic pictures depend upon the stage and degree of the process. The arterial calcification occurs in relation to the internal elastic lamina, and its pattern is determined by the course of that membrane. This relation is shown by study of the earliest lesions;

in the later stages the lamina may be destroyed and its significance no longer apparent.

The various processes which may occur in the course of calcification of coronary and other arteries in infants include the following (not all may be observed in any one case): (1) The normally eosinophilic internal elastic lamina is interrupted by regions in which there have been deposited fine basophilic granules of calcium salts. (2) The internal elastic lamina may preserve its continuity as an eosinophilic hyaline band, with a fine deposit of basophilic material either on both sides of and closely apposed to it, or on the medial side only. (3) A loss of continuity of the internal elastic lamina is first observed where calcareous granules are in contact with it. (4) The internal elastic lamina may maintain its identity and be seen as a continuous or interrupted eosinophilic membrane either on the inner (intimal) border of deposited calcium salts or coursing through the midst of a large basophilic mass. (5) The internal elastic lamina may not be identifiable and only large clumps or broad bands of calcium salts be seen. (6) The larger calcific masses on the medial side of the internal elastic lamina occupy the area formerly filled with the muscular and elastic fibers of the media. It is believed that these fibers atrophy and are replaced by the deposits of lime salts. (7) An intimal fibroblastic reaction is present, beginning with the first fine basophilic deposits. Occasionally the calcific process in the vicinity of the internal elastic lamina extends into this fibrous tissue. (8) A giant cell reaction, of foreign body type, may be seen around some of the masses of lime salts.

Since the internal elastic lamina is morphologically a part of the intima of the artery, it may be questioned whether the term "medial calcification" should be applied to this process as most authors have done. When the calcareous material is deposited along the medial aspect of the lamina it may simulate a deposit in the media. This appearance is interpreted to be due to a previous atrophy of medial fibers and replacement of them by masses of calcium salts originating along the internal lamina. In nearly all previously reported cases of coronary calcification in infants, this relation of the calcium deposits to the internal elastic lamina, in at least the early stages, has been described.

This report is concerned chiefly with the process of calcification as it affects the coronary arteries. It should be noted, however, that these arteries are not the only vessels affected. In nearly all previously reported cases and in the 5 described here, there were arteries in other organs which showed lesions more or less severe than those in the heart. In some cases the effects upon organs supplied by these ves-

sels have been of principal clinical importance. Instances of calcification of similar type in the arteries of infants in whom the coronary arteries were not involved have been reported.<sup>9</sup> Probably the causal factors are similar.

### *Possible Causal Factors*

The similarity of the vascular lesions and marked calcification in this type of infantile coronary calcification to those found in lesions known to occur in diseases with abnormal concentrations of serum calcium and phosphorus suggests possible causes.

1. *Renal Lesions.* In several previously reported cases renal lesions have been found. In some instances these lesions have been marked and of undoubted long duration. It is probable that these cases are actually examples of so-called calcium gout or of renal dysfunction with an altered calcium-phosphorus ratio and subsequent metastatic calcification. The term "renal rickets" has been applied to this disorder, although the osseous lesions of rickets have in some instances been minimal or lacking. Microscopic study of calcified arteries in other cases of renal rickets<sup>14,15</sup> has shown the calcification to occur in relation to the internal elastic lamina in early lesions and later to involve apparently the entire media. Thus the process is similar to that described in this report. Renal lesions of extent sufficient to consider them a primary factor in arterial calcification were present in cases reported by Bryant and White,<sup>2</sup> Lightwood,<sup>8</sup> and Andersen and Schlesinger,<sup>13</sup> and it is probable that the cases of Surbek,<sup>3</sup> Forrer,<sup>6</sup> and van Creveld<sup>11</sup> should likewise be included. The description of the renal lesions in these latter cases is incomplete. In 3 cases of the first group (Lightwood,<sup>8</sup> Andersen and Schlesinger<sup>13</sup>) in which chemical studies of the blood were made, abnormal calcium-phosphorus values were found. In case 4 of the present series, polycystic kidneys were present; this infant was stillborn, and the significance of such renal lesions in the extremely young has not been established.

The calcific lesions found in the kidneys in others cases may well be only another manifestation of the process causing the general arterial changes. In most of the instances in which calcification of tubular epithelium or of glomeruli has been found, there has been calcification also of the arteries of the affected kidneys. Unfortunately, no chemical studies of the blood are available for this group so that the degree of renal dysfunction is not known with certainty. That renal dysfunction is not the only etiologic factor, however, is shown by the occurrence of calcification in patients in whom no renal lesions were present.

2. *Lesions of the Parathyroid Glands.* Altered concentrations of

serum calcium and serum phosphorus may be due also to primary parathyroid hyperplasia or to a parathyroid tumor. Excluding those cases in which parathyroid hyperplasia is probably secondary to a renal lesion, the so-called renal hyperparathyroidism, the state of these glands is known only in the case reported by Iff.<sup>7</sup> No abnormality was found in that case. The morphologic appearances and the distribution of the calcific lesions in some cases of coronary calcification in infants are very similar to those in known cases of primary parathyroid hyperfunction. It is unfortunate that the parathyroid glands were not examined in the earlier cases, or in those reported here.

3. *Primary and Destructive Diseases of Bone.* Primary bone diseases, such as osteogenesis imperfecta, and destructive bone diseases, such as may occur with neoplastic metastasis, may be associated with altered concentrations of serum calcium and phosphorus. Calcification of arteries other than the coronary arteries has been reported in osteogenesis imperfecta<sup>16</sup> and in generalized osteitis fibrosa.<sup>17</sup> The bones were examined in only 3 of the cases of infantile coronary calcification reported in the literature. Changes were found in only one case<sup>8</sup> where the renal lesion and associated parathyroid hyperplasia furnished an explanation for the abnormalities. The few sections of bone available in my cases showed no change.

4. *Hypervitaminosis D.* A fourth cause of calcific lesions in arteries and tissues is hypervitaminosis D. This condition is also associated with altered serum values of calcium and phosphorus. Vitamin D was given to four of the infants whose lesions have been reported elsewhere. In 2 cases the amount was not excessive;<sup>8,10</sup> in 2 (Andersen and Schlesinger<sup>13</sup>) the dosage was larger than usual, up to four times the regular amount. The administration of vitamin D, however, is denied in many reports while other cases antedate the introduction of this product. No vascular lesions have been seen in many instances in which huge dosages have been given.<sup>18</sup> The experimental work of Goormaghtigh and Handovsky<sup>19</sup> with dogs indicates that the initial lesion in the calcification associated with hypervitaminosis D is degeneration of smooth muscle of the media. Such calcification is dystrophic in type. An increased sensitivity to a mild excess of vitamin D or calcium in cases of renal rickets may explain the frequency with which calcification is seen in that disease,<sup>20</sup> and it may be that in some persons there is an abnormal response even in the absence of renal lesions. In case 1 of the present series overdosage with vitamin D was originally suspected as a cause of the lesion, but the physician in charge was unable to find any known use of vitamin D in the family.

5. *Infection.* Factors other than altered calcium-phosphorus bal-

ance must be considered. Damage of the arteries may be the result of infection. Degeneration of the media, which may be severe enough to include necrosis and destruction of elastic fibers, has been reported.<sup>21</sup> to follow certain toxic or infectious diseases, and calcification of the internal elastic lamina has been noted.<sup>22</sup> In 3 cases (Surbek,<sup>3</sup> Forrer,<sup>6</sup> and Lightwood<sup>8</sup>) there were coincident infective lesions of various organs, and Iff<sup>7</sup> believed that the mother of his patient had a low-grade infection during the period of gestation. In 3 of the cases here presented purulent pneumonitis was found, but in each this was a terminal feature and therefore probably was not related to the vascular lesions. The evidence in favor of infection as the primary cause in all cases of arterial calcification is slight, and there are multiple instances of intrauterine and neonatal infection without coexistent or subsequent arterial calcification. In some cases, however, it may be a contributing factor. Syphilis has also been implicated. In 2 of the 20 cases a diagnosis of syphilis was made either as a result of serologic examination of the patient or of the mother, or on the basis of tissue changes. In 10 cases, serologic examinations gave negative results. Again the evidence does not include all cases, but calcification is not a process generally considered to result from syphilitic infection. Search for spirochetes in the cases in which a diagnosis of congenital syphilis had been made was not successful.

6. *Allergy.* Allergy as the cause of arterial lesions was suggested by van Creveld.<sup>11</sup> He reported 2 cases, in the first of which the mother was asthmatic and had used caffeine and lobeline during the period of gestation. The suggestion was made that the lesions in the infant could be attributed to these drugs with probable damage to the vessel wall. Proof was attempted by animal experimentation, but there was no success. In his second case there was a familial history of allergic disease.

7. *Other Nonspecific Calcific Lesions of Arteries of Similar Pattern.* The dystrophic calcification of arteries in the senile uterus appears first in relation to the internal elastic lamina, but the arteries affected possess a thick hyalinized intima and there is occasionally fibrous tissue in the media. Such changes were not seen in the arteries of the infants. In sclerosis of Mönckeberg's type the calcific deposit is in the media, and the internal elastic lamina acts only as a limiting membrane.

Adult arterial calcification with a histologic appearance closely resembling the calcification of coronary arteries in infants is found in the medium-sized pericapsular arteries of the thyroid gland. This process has been designated "isolierte Verkalkung der Membrana elastica interna";<sup>23</sup> it may occasionally be seen in arteries in other organs. Only a few of the arteries of a gland may be affected. The deposit is again



in relation to the internal elastic lamina; it may be on both sides or only on the medial side of the membrane; the internal elastic lamina may be continuous through the deposit or may be interrupted; and when calcification is marked the masses occupy the media, with atrophy and replacement of the medial fibers (Fig. 13). Thus it is comparable to the process described in this report. Fibroblastic proliferation of the intima also may occur. These lesions are frequently seen in young persons.

8. *Rôle of Elastic Tissue.* The rôle of elastic tissue in the pathogenesis of calcific lesions in arterial walls may be important. Wells<sup>24</sup> noted that "elastic tissue seems prone to an early calcification, and it is not uncommon to see the elastic laminae of small arteries calcified in an apparently selective manner." Elastic fibers have been observed to be susceptible to easy damage by toxins, drugs, and other agents.<sup>22</sup> Such exogenous factors may not be necessary. It is unfortunate that no post-mortem examination was made of the sibling of the patient described as case 1, who was reported by the family physician to have died with a similar clinical picture. The primary etiologic feature in all these cases may be congenital weakness of the elastic tissue of the arterial walls. Such a weakness has been postulated as a main factor in the development of arteriosclerosis of the adult type.<sup>24</sup> Chronic illness of the mother might be a cause of this weakness in the child; thus syphilis, allergic diseases, bronchiectasis, and other processes described in the mothers of the various infants might exert a common effect on the offspring. When calcification of this type has occurred in infants in arteries other than coronary arteries, various maternal diseases have been reported by which the elastic tissue could be similarly affected. Acting on such an imperfect ground substance, a variety of factors may cause additional degeneration and subsequent calcification. Although renal disease in some children is associated with arterial calcification, this is not true of all. Similarly, not all patients with hyperparathyroidism nor all with primary bone disease show vascular lesions. The dose of vitamin D which is toxic and which may lead to degenerative lesions may vary with the constitution of the individual. Lippincott<sup>25</sup> has reported a case of possible hypervitaminosis D in an infant, 10 months old; a twin brother (with probable similar biologic characteristics) exhibited symptomatology to a less degree. When, as in experimental animals, dosages beyond the general toxic levels are given, even the best tissue may be affected. Likewise, other dietary factors, infection, or any other extrinsic or intrinsic factor may be the precipitating mechanism which can act upon the embryonally weak protoplasm. This possibility as a cause of the calcification, although the

most attractive, requires experimental demonstration for complete acceptance.

### SUMMARY

Five cases of calcification of the wall of the coronary arteries of infants, with associated fibroblastic proliferation of the intima, have been described. The calcification occurs in relation to the internal elastic lamina. In one case the lesion was the probable cause of death; in three others it may have been a contributing factor. Various possible etiologic factors have been discussed. It is considered most probable that no single etiologic factor is present other than an embryonally defective elastic tissue in the arterial walls, upon which various factors can act to cause calcification. Such calcification with associated fibroblastic proliferation of the intima is the most frequent cause of coronary occlusion in infants.

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## DESCRIPTION OF PLATES

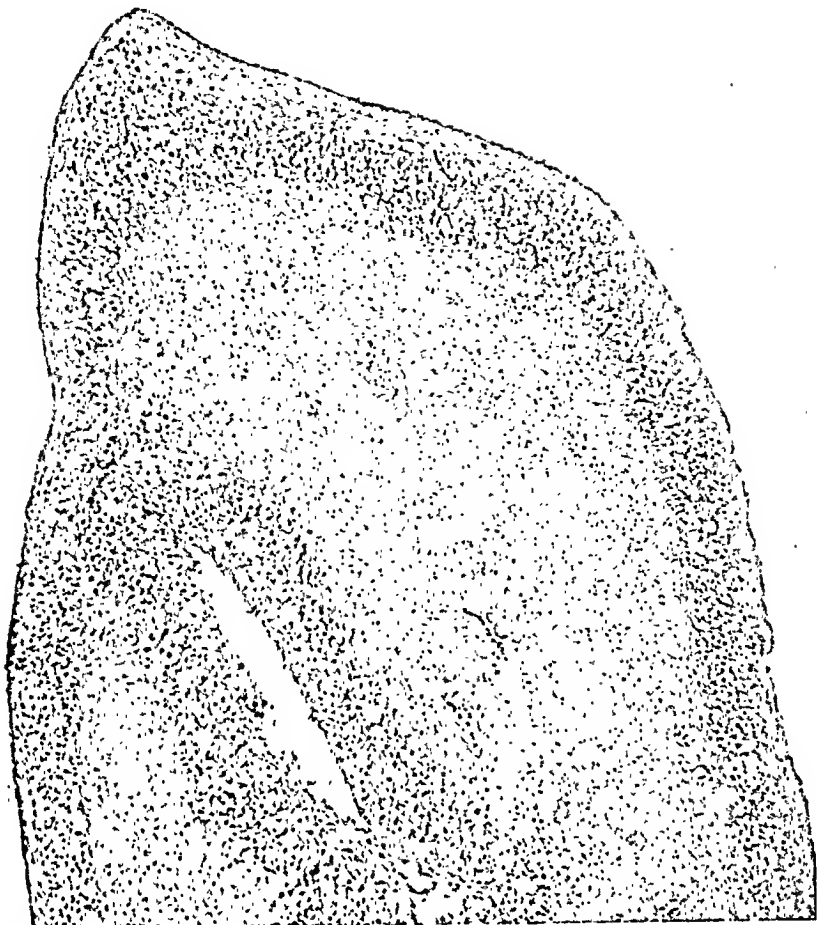
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### PLATE 191

FIG. 1. Case 1. (Age, 3 months.) Infarction of the central portion of a papillary muscle. Hematoxylin and eosin stain.  $\times 100$ .

FIG. 2. Case 1. Coronary artery showing calcification of the internal elastic lamina and adjacent inner media. Fibroblastic proliferation of the intima with newly formed blood-channels. Hematoxylin and eosin stain.  $\times 150$ .

1



2



### PLATE 192

- FIG. 3. Case 1. (Age, 3 months.) Focal calcification of the internal elastic lamina and adjacent tissues on both sides in a small artery at the hilum of a kidney. Hematoxylin and eosin stain.  $\times 350$ .
- FIG. 4. Case 2. (Age, 2 months.) Localized calcification of the wall of the splenic artery. (The section was focused so as to emphasize the internal elastic lamina.) Hematoxylin and eosin stain.  $\times 60$ .
- FIG. 5. Case 2. Higher magnification of a localized area of calcification in the same splenic artery. The internal elastic lamina can be identified in the midst of the calcium. Hematoxylin and eosin stain.  $\times 230$ .



4

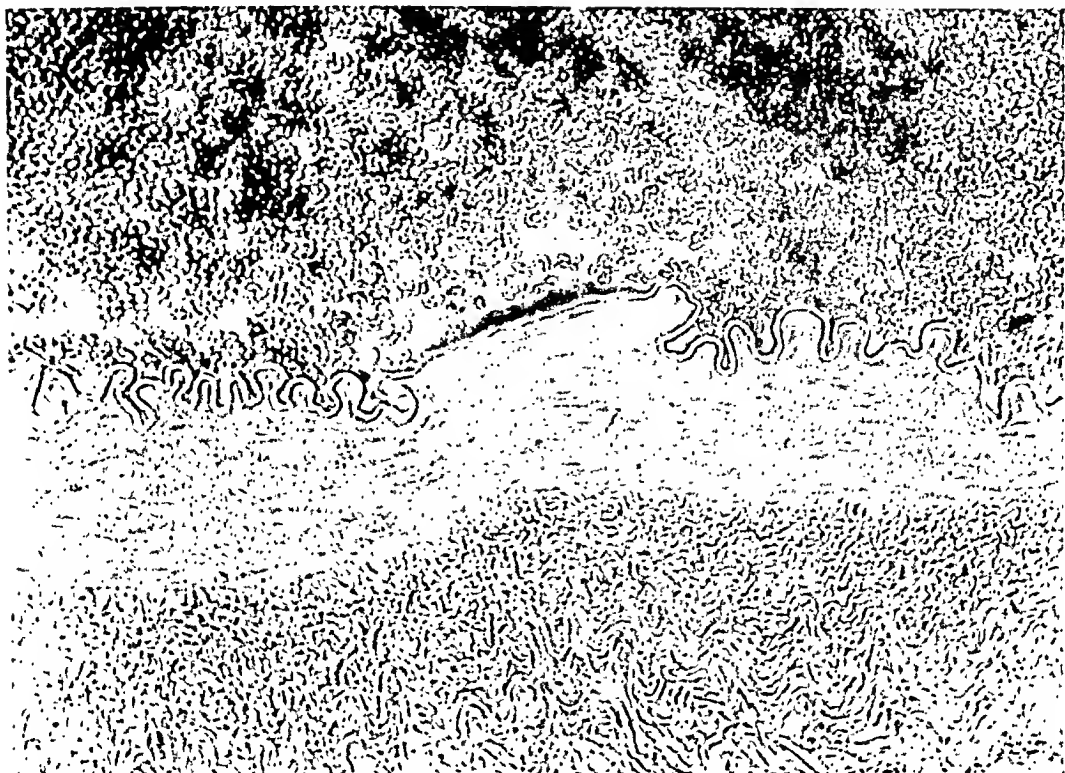
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Stryker

Coronary Arterial Calcification in Infancy

PLATE 193

- FIG. 6. Case 2. (Age, 2 months.) Higher magnification of another portion of the splenic artery used for Figures 4 and 5, showing deposition of calcium on the inner side of the internal elastic lamina. (The section was focused so as to emphasize the internal elastic lamina.) Hematoxylin and eosin stain.  $\times 225$ .
- FIG. 7. Case 3. (Age, 6 months.) Coronary artery showing calcification along the course of the internal elastic lamina. Focal interruption of continuity of the lamina. Fibroblastic proliferation of the intima. Hematoxylin and eosin stain.  $\times 50$ .



6



7



PLATE 194

FIG. 8. Case 3. (Age, 6 months.) Artery in periadrenal connective tissue showing marked calcification in the region of the internal elastic lamina; the lamina cannot be identified. Marked fibroblastic proliferation of the intima with nearly complete occlusion of the lumen. Hematoxylin and eosin stain.  $\times 100$ .

FIG. 9. Case 4. (Stillborn.) Focal calcification on both sides of elastic fibers in the pulmonary artery. Fibroblastic proliferation of the intima. Hematoxylin and eosin stain.  $\times 30$ .

8



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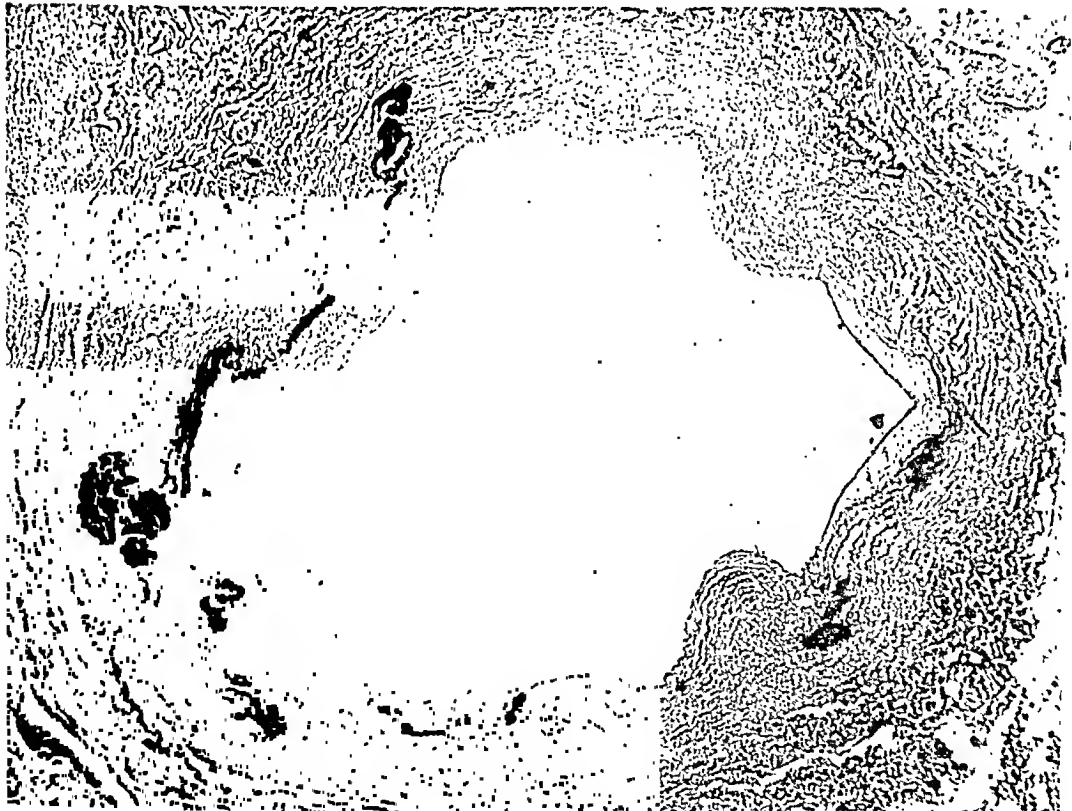
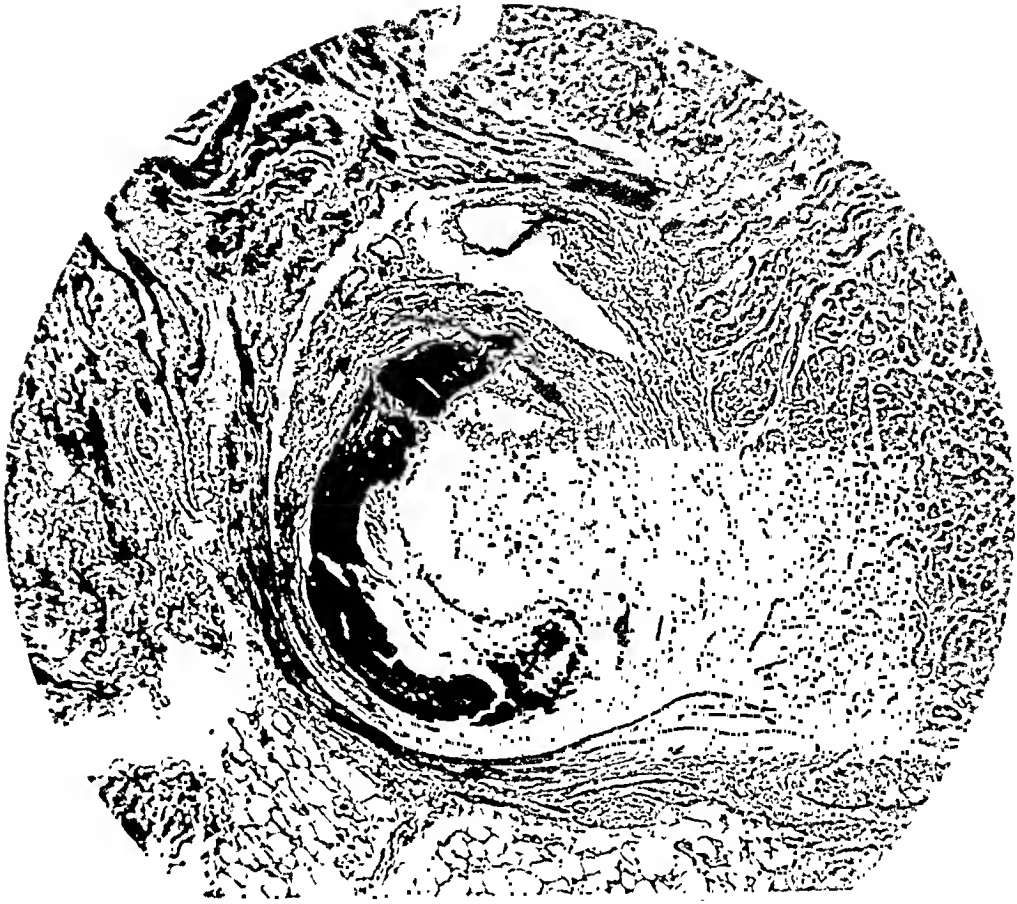


PLATE 195

- FIG. 10. Case 5. (Age, 7 months.) Coronary artery with calcification of the internal elastic lamina and of the tissues on both sides of it. Fibroblastic proliferation of the intima. Hematoxylin and eosin stain.  $\times 100$ .
- FIG. 11. Case 5. Marked calcification of the wall of a pancreatic artery. Fibroblastic proliferation of the intima in the region of calcification. Hematoxylin and eosin stain.  $\times 100$ .

10



11

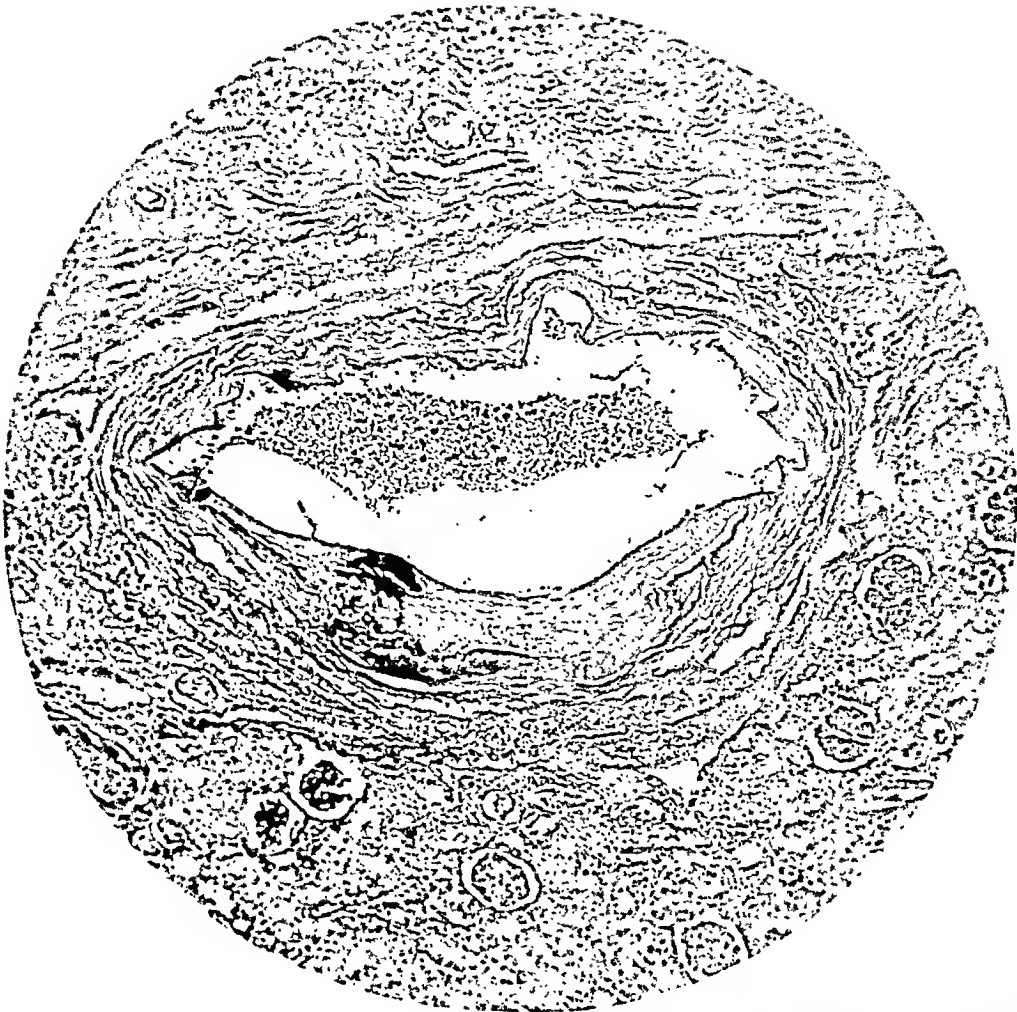


PLATE 196

FIG. 12. Case 5. (Age, 7 months.) Focal calcification along the course of the internal elastic lamina of a renal artery. Fibroblastic proliferation of the intima. Hematoxylin and eosin stain.  $\times 100$ .

FIG. 13. (Middle-aged adult.) Calcification of the internal elastic lamina of a pericapsular artery of the thyroid gland. Small deposits of calcium on both sides of the lamina. Fibroblastic proliferation of the intima. Hematoxylin and eosin stain.  $\times 30$ .

12



13





# SKELETAL CHANGES CAUSED BY THE COMBINED ADMINISTRATION OF THYROXIN AND ESTROGEN \*

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In growing animals, both estrogenic and thyroid hormones accelerated skeletal ageing.<sup>1,2</sup> However, the two hormones exerted different effects on the three phases of the skeletal time curve.<sup>1,3</sup> Thyroxin promoted only to a minor degree the first phase, that of growth; it hastened the onset and progress of the second phase during which degeneration of cartilage is predominant; it stimulated, in particular, the third phase during which resorption of cartilage and bone leads to epiphyseo-diaphyseal union. Estrogen, on the other hand, suppressed growth, accentuated degeneration, and markedly inhibited resorptive processes. When applied simultaneously, the two hormones might neutralize each other, or a combination effect might ensue as was seen after combined administration of anterior hypophyseal and estrogenic hormone.<sup>4</sup> Under the latter conditions, each hormone tended to exert its own action on cartilage and bone: The growth inhibition caused by estrogen, when given singly, was decreased, but there was an intensification of the ageing of cartilage and of ossification over that caused by either hormone alone. The present investigation was undertaken in order to study a possible modification of the estrogen effect by thyroxin.

## MATERIAL AND METHODS

Sixty-three virgin mice of the closely inbred strain C57 black, 5 to 6 weeks old, kept on a standard diet of Purina Dog Chow and water, were used in these experiments. The mice were divided into the following five groups: Group I: Twenty-six animals were injected subcutaneously with 100 rat units of alpha estradiol benzoate in sesame oil once a week and with 0.1 mg. of thyroxin three times a week.<sup>†</sup> Four mice each were thus treated for 1, 2, 4, or 8 weeks, and 2 animals each for 4, 8, 10, 12, or 14 months. Group II: Nine mice received estrogen only for periods corresponding to those in Group I. Group III: Nine animals were injected with thyroxin alone. Group IV: Nine mice remained as untreated controls. Group V: Ten animals were given 200 rat units of the estrogen once a week for 4 weeks. Thereafter the estrogen treatment was discontinued and 5 of these mice were injected

\* Aided by the Louis M. Monheimer Memorial Fund.

Received for publication, September 17, 1945.

<sup>†</sup> We are indebted to Dr. Erwin Schwenk of the Schering Corporation for a generous supply of Progynon-B and thyroxin.



with 0.1 mg. of thyroxin three times weekly for 1, 2, 4, 6, or 8 weeks. At the end of each period, one mouse was sacrificed together with one control that had not received thyroxin.

In all animals the lower end of the femur and the upper end of the tibia were removed as a whole, and sections prepared for histological studies.

## HISTOLOGICAL EXAMINATION

### *A. Epiphyseal Disk*

Under the combined influence of estrogen and thyroxin, the age changes in the growth zones at any given stage were markedly advanced over those found in untreated animals. After 1 or 2 weeks of treatment, the epiphyseal disks were narrower and more heavily calcified than ordinarily. Although the columnar and hypertrophic cartilage cells were only slightly less numerous than usual, they were distinctly smaller. Thus the cartilage cell rows were shorter than in untreated mice of the same age. Under the influence of estrogen alone, the cartilage cells were not only markedly decreased in size but also in number. On the other hand, after injections of thyroxin only, the conversion of the proliferating columnar cartilage into hypertrophic cartilage was intensified.

These changes were even more pronounced when the hormones were allowed to act for 8 weeks. In Figures 1 to 4, the growth zones of 3-months-old untreated and treated mice are shown. In the control animal (Fig. 1), the individual cartilage cell rows were regularly arranged, separated from one another by thin layers of ground substance and composed of eight columnar and two or three hypertrophic cartilage cells. In the animals receiving both hormones (Fig. 2), the matrix was more abundant and hyalinized, the cell rows were scarcely half as high as ordinarily, calcification as well as destruction of cartilage was marked, and amorphous plugs of disintegrated or ossified cartilage cell rows were noted. In untreated mice, a comparable condition was present at about the age of 6 months. In animals injected for the same length of time with corresponding doses of estrogen only (Fig. 3), calcification of the epiphyseal cartilage was accentuated and the proliferation and hypertrophy of the cartilage cells were decreased to a greater extent. Under the influence of thyroxin alone (Fig. 4), resorption of the cartilage was more marked than after the combined administration of both hormones.

In prolonged experiments, the differences between control and experimental animals became still more conspicuous. In Figures 5 to 8, the findings in 7½-months-old control mice and in animals treated for 6 months are demonstrated. In untreated mice (Fig. 5), the growth

zone still consisted of a continuous layer of calcified cartilage containing thick amorphous plugs of destroyed cartilage. Under the influence of both hormones (Fig. 6), the zones of endochondral ossification had been converted into a thin bony scar showing some remnants of calcified cartilage. In noninjected animals, a similar condition was not observed before the end of the second year of life. In mice receiving only estrogen (Fig. 7), the epiphyseal disk was composed of a layer of densely calcified, inactive cartilage showing little resorption. After treatment with thyroxin alone (Fig. 8), a thin, osseous lamella had taken the place of the former growth zone, and there were wide communications between epiphysis and diaphysis. The resorptive processes thus surpassed those noted under the combined influence of both hormones.

Simultaneous treatment with both hormones for 12 months and more led to complete epiphyseo-diaphyseal union. Such an advanced degree of skeletal development was observed likewise after injections of thyroxin alone, but not in untreated old mice nor in those receiving estrogen only.

### *B. Diaphysis*

In mice treated with estrogen and thyroxin simultaneously for 1 or 2 weeks, the metaphysis contained more fibrous tissue and fewer capillaries than ordinarily. There was little replacement of the hypertrophic cartilage by bone; the osseous spicules were broader, firmer, and longer than usual, and here and there they were linked with one another and the shaft by bony bridges. The shafts were thickened and the vascular canals narrowed; the periosteal and endosteal connective tissue was dense and contained abundant collagenous fibers.

In noninjected mice 3 months of age, the replacement of cartilage by bone was progressing actively (Fig. 1). In animals of corresponding age treated with both hormones for 2 months (Fig. 2), a transverse bony lamella, here and there corroded by advancing capillaries, delimited the epiphyseal cartilage from the metaphysis. The spicules were thick but in some places resorbed by bone marrow; the interlacing of the trabeculae was less conspicuous than previously. The vessels of the shaft were enlarged and the periosteal and endosteal connective tissue was loosened. Under the influence of estrogen alone (Fig. 3), thick interlacing bone filled the proximal part of the marrow cavity. The osseous shaft was heavily calcified and showed narrowed vessels; the periosteal and endosteal connective tissue was denser than usual and poorly vascularized. In animals receiving only thyroxin (Fig. 4), the primary trabeculae were resorbed; the bone marrow was rich in capillaries, and the vessels perforating the shafts were enlarged.

After 6 months of combined treatment with both hormones, much of the excessive bone had been dissolved; the diaphysis again contained hemopoietic marrow (Fig. 6), and only fragments of small spicules. The compacta of the shaft was thin and showed numerous congested vessels. After injections of estrogen alone (Fig. 7), resorption of bone, although indicated by the formation of fibrous tissue along the trabecular network, was by far less active than after the simultaneous injections of both hormones. By contrast, in animals receiving only thyroxin (Fig. 8), the diaphysis was cleared of all primary bone; the marrow contained enlarged capillaries; the shafts were thinner than ordinarily; the vessels perforating the shaft were enlarged; and the periosteal connective tissue was loose.

In mice injected for 12 months and more with both hormones, all trabecular bone had been removed and the shafts were thinner than in untreated animals. The conditions thus resembled those seen subsequent to the administration of thyroxin alone for the same period, whereas in animals treated with estrogen only, the metaphysis still was delimited from the epiphysis by a bony plate. Moreover, strands of fibrous tissue indicated that hyperossification had taken place at some earlier date.

#### CHANGES CAUSED BY ESTROGEN AND SUBSEQUENT ADMINISTRATION OF THYROXIN

When thyroxin was given following treatment with estrogen for 4 weeks, the age changes in the cartilage, already accelerated and intensified by the estrogen injections, were further increased. Plugs of disintegrated cartilage cells appeared in the greatly narrowed growth zones. After 8 weeks of treatment with thyroxin, the resorptive processes were so pronounced as to produce perforations of the epiphyseal plates.

In order to evaluate the part played by thyroxin in these effects, they were compared with conditions in mice that had received estrogen for 4 weeks, but no further treatment. In these animals, proliferation and hypertrophy of the epiphyseal cartilage were resumed, the growth zones again became wider and the matrix looser. In spite of the resumption of the growth processes, however, the histological age of the epiphyseal cartilage in 4-months-old mice was advanced about 3 months over that seen in untreated animals. On the other hand, the epiphyseal plate of the corresponding mouse treated in addition with thyroxin resembled that of an untreated animal toward the end of the first year of life.

In the metaphysis, thyroxin given subsequent to estrogen accelerated the resorptive processes as compared with those in the mice receiving

no thyroxin; 4 and 8 weeks after discontinuation of the estrogen injections there was still a thick layer of subepiphyseal bone and of thick and dense spicules in the marrow cavity; however, an interlacing osseous network was no longer noticeable. Under the additional influence of thyroxin, a good deal of the excessive bone had disappeared after 4 weeks of treatment, and after 8 weeks the subepiphyseal bony plate was reduced to a thin lamella and the marrow cavity was cleared of all trabecular bone. The intensified resorption was also indicated by the more frequent appearance of multinucleated giant cells and by increased vascularization of the connective tissue and the shafts.

### DISCUSSION

The findings in the skeleton of (a) untreated mice, and the changes produced by (b) estrogen, (c) thyroxin, and (d) the combined administration of these two hormones are schematically presented in Tables I and II.

Table I summarizes the findings after 2 months of observation. The combined action of thyroxin and estrogen manifested itself as follows: The addition of thyroxin did not materially affect the decrease in the proliferation of the cartilage caused by estrogen; however, it partly counteracted the marked inhibition of hypertrophy and resorption of the cartilage called forth by estrogen. Under the combined influence of these hormones, degeneration of cartilage was intense; on the other hand, the metaphyseal connective tissue was less hyalinized, and the bone present was less abundant and more loosely knit than after injections of estrogen only.

Table II demonstrates the findings after 6 months of observation. When both hormones were given, ossification and particularly resorption of the epiphyseal cartilage resembled closely the same processes seen after administration of thyroxin alone; they were advanced over the normal, although less so than under the sole influence of thyroxin. Likewise, the resorption of bone in the shaft and metaphysis was more conspicuous after administration of both hormones than after that of estrogen alone.

Correspondingly, when thyroxin was allowed to act subsequent to the treatment with estrogen, resorption was resumed more rapidly and it proceeded with greater intensity than in animals receiving no further injections. However, this reversal of the estrogen effect concerned primarily the changes in the bone; in the cartilage, age changes due to thyroxin were added to those caused previously by the estrogen.

When administered simultaneously, the two hormones competed with each other in enforcing their characteristic effects on the skeletal time curve. Each hormone predominated during that phase in which

TABLE I

SCHEMATIC PRESENTATION OF SKELETAL FINDINGS IN NORMAL AND EXPERIMENTAL MICE AFTER 2 MONTHS OF OBSERVATION				
	(a) <i>Untreated</i>	(b) <i>Estrogen</i>	(c) <i>Thyroxin</i>	(d) <i>Estrogen and thyroxin</i>
Epiphyseal cartilage	Proliferation: in progress	decreased	ceased	decreased
	Hypertrophy: in progress	decreased	ceased	somewhat decreased
	Degeneration: absent	marked	marked	marked
	Resorption: in progress	greatly decreased	increased	somewhat decreased
Metaphysis	Connective tissue: loose, vascular	hyalinized; vascularization greatly decreased	vascularization increased	vascularization and hyalinization decreased
	Bone: thin trabeculae	resorption greatly decreased; inter-lacing thick bone	trabecular bone resorbed	resorption somewhat decreased; moderately thick trabeculae

TABLE II

SCHEMATIC PRESENTATION OF SKELETAL FINDINGS IN NORMAL AND EXPERIMENTAL MICE AFTER 6 MONTHS OF OBSERVATION				
	(a) <i>Untreated</i>	(b) <i>Estrogen</i>	(c) <i>Thyroxin</i>	(d) <i>Estrogen and thyroxin</i>
Epiphyseal cartilage	Degeneration; resorption and ossification in progress	inactive; calcific; little resorption	resorbed; epiphyseal diaphyseal union	scanty remnants of inactive cartilage in thin bony scar
	Hemopoietic marrow; trabeculae resorbed	some inter-lacing of trabeculae	hemopoietic marrow; trabeculae resorbed	hemopoietic marrow; remnants of bony spicules
Metaphysis	Moderately thick	thickened; vascularization decreased	thinned; vascularization increased	thin; vascular
Shaft				

it acted most conspicuously when injected singly. During the first phase the inhibition of proliferation of cartilage caused by estrogen was not counteracted by thyroxin; however, there was an effective antagonism on the part of thyroxin as to the hypertrophy of cartilage; during the second phase, thyroxin did not diminish the degeneration nor the calcification of the cartilage called forth by estrogen; during the third phase, the influence of thyroxin became predominant, and the aged cartilage responded readily to the resorptive action of thyroid hormone.

Thus, in spite of their antagonistic activities, thyroxin and estrogen did not neutralize each other in respect to their skeletal effects in growing mice. On the other hand, in chickens<sup>5</sup> and pigeons<sup>6</sup> receiving equal amounts of the two hormones, the rise of serum calcium, phosphorus, and cholesterol occurring under the influence of estrogen was prevented; but in pigeons<sup>6</sup> thyroxin given at this ratio did not measurably modify the medullary bone formation called forth by estrogen. Our mice received six times as much thyroxin as estrogen. It is possible that in other species also, the skeletal effects of estrogen might be modified by increasing the dose of thyroxin.

Until more accurate data are established concerning the effects of thyroxin and estrogen respectively on local tissue metabolism and vascular activity, no definite correlation seems possible between such effects and the morphological changes in the skeleton. The scanty and contradictory data, however, suggest that such correlations do exist.<sup>7</sup> Histologically, the antagonism of these two hormones is more conspicuous in tissue near vessels, such as the metaphysis and shaft; it is less obvious or even absent in the nonvascularized cartilage. Enlarged capillaries—a thyroxin effect—have greater resorptive power due perhaps to an increased permeability of their walls. Conversely, a coat of unyielding hyaline material as well as of fibrous tissue<sup>1,8</sup>—an estrogen effect—presents an obstacle to the processes of resorption; in addition, it may decrease the ability of the capillaries to dilate. Furthermore, increased vascularization may allow a more active exchange of substances between blood and tissues and thus prevent the accumulation of inert material such as hyalin. However, the two hormones might also affect directly the metabolism of the skeletal tissues. In mice, thyroxin markedly increases the turnover of radioactive phosphorus.<sup>9</sup> Estrogen, on the other hand, decreases, at least temporarily, the water content of heart and muscle, pancreas, and brain.<sup>10</sup> A similar effect on the bone marrow would be quite compatible with the structural changes produced by estrogen. The percentage of water in bone itself is decreased<sup>11</sup> under the influence of estrogen.

## SUMMARY

In growing mice, the skeletal effects of estrogen can be modified but not prevented by simultaneous administration of thyroxin. Thyroxin partly restores both hypertrophy of cartilage and resorption of cartilage and bone which are markedly inhibited by estrogen. However, thyroxin does not counteract the suppression of proliferation of the cartilage and its regression caused by estrogen. The interaction of the two hormones results in a combination effect, with estrogen predominating during the earlier, and thyroxin during the later, stages of administration.

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## DESCRIPTION OF PLATES

## PLATE 197

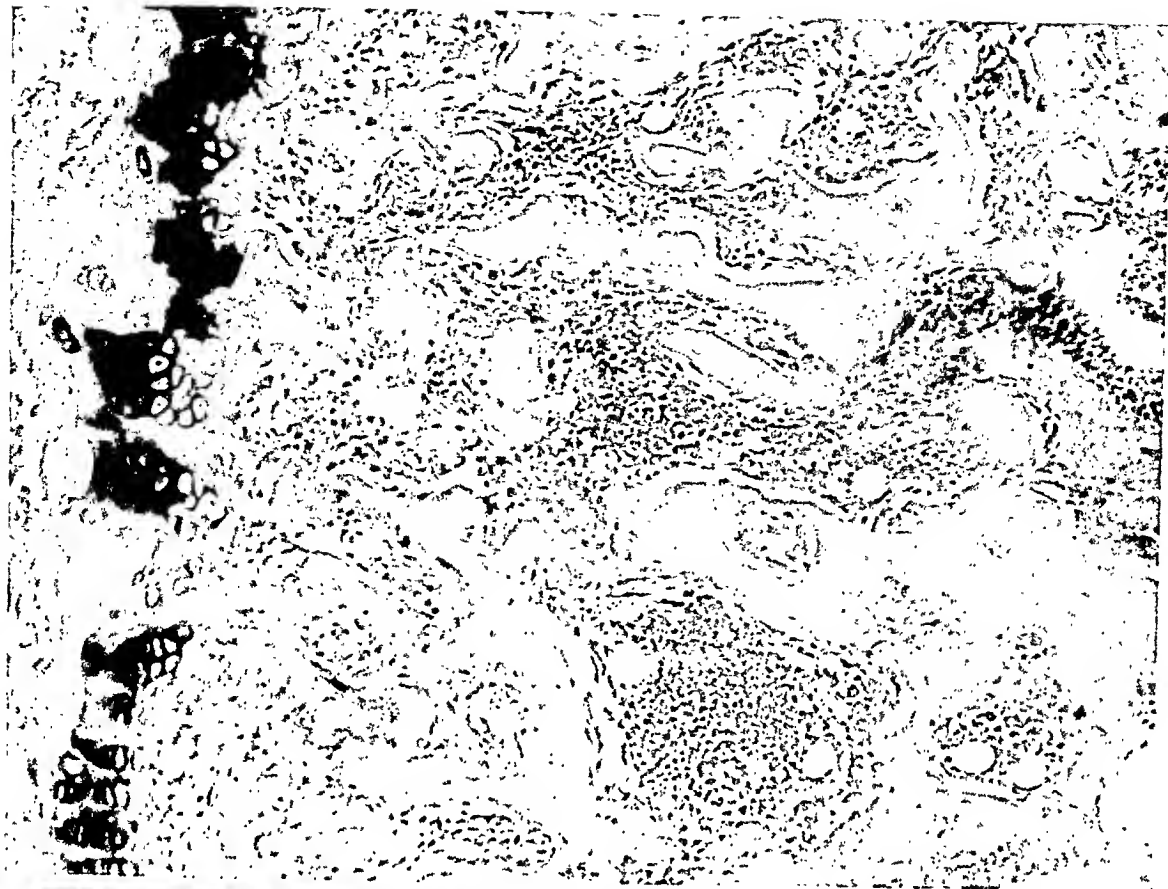
FIG. 1. Growth zone at the upper end of the tibia of an untreated mouse of strain C57, 3 months old. The cartilage columns show regular configuration, the primary spicules are long and delicate.  $\times 170$ .

FIG. 2. Growth zone at the upper end of the tibia of a mouse of strain C57, 3 months old, which had received 100 rat units of estrogen once and 0.1 mg. of thyroxin three times weekly for 8 weeks. The epiphyseal plate is narrowed, and growth of cartilage is markedly decreased. There is a large osseous plug present in the growth zone. The primary spicules are longer and thicker than in Figure 1, but not as thick and interlaced as in Figure 3.  $\times 170$ .



1

Silberberg and Silberberg



2

Thyroxin and Estrogen



PLATE 198

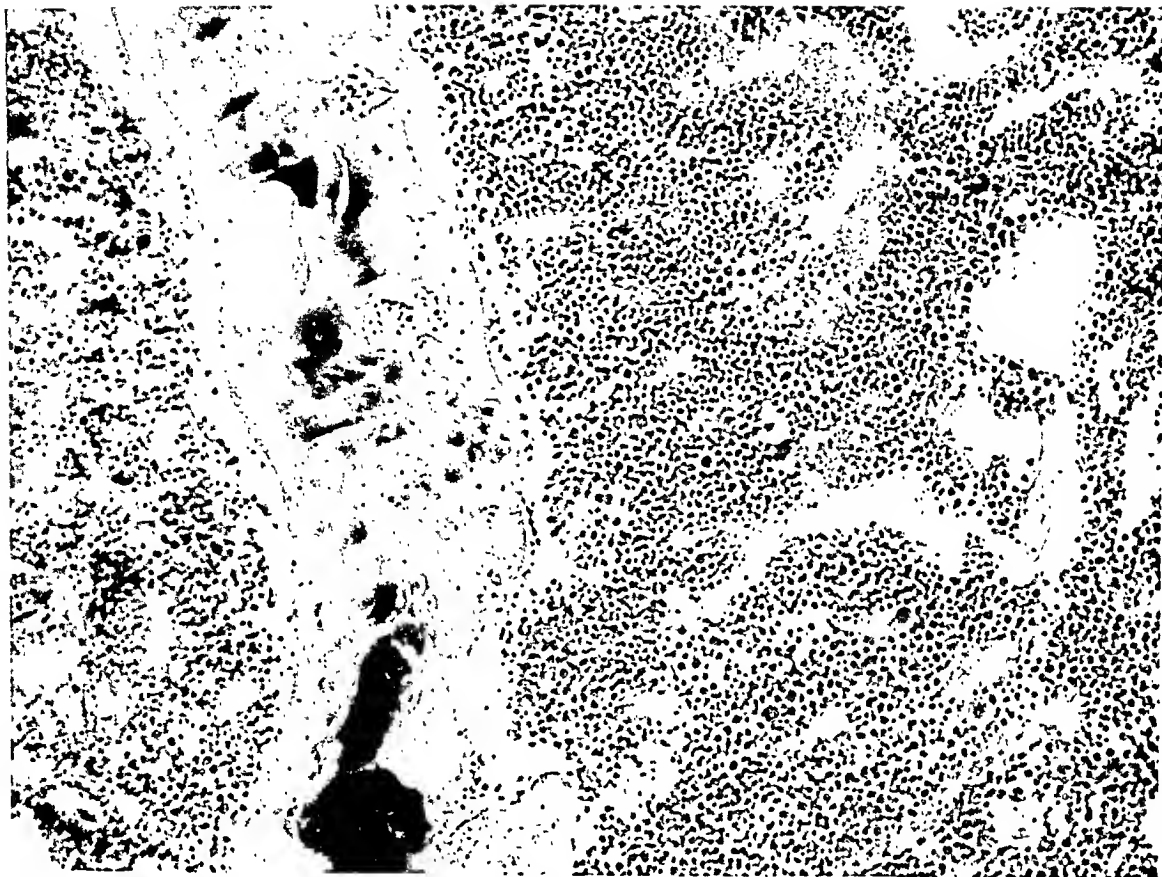
FIG. 3. Growth zone at the upper end of the tibia of a mouse of strain C57, 3 months old, which had received 100 rat units of estrogen once a week for 8 weeks. The layer of cartilage is somewhat narrower than in Figure 2. There are no hypertrophic cartilage cells present. Calcification and plug formation are of about the same order as in Figure 2. Dense interlacing bone is found in the metaphysis.  $\times 170$ .

FIG. 4. Growth zone at the upper end of the tibia of a mouse of strain C57, 3 months old, which had received 0.1 mg. of thyroxin three times weekly for 8 weeks. Growth of cartilage has ceased as indicated by a solid osseous plate underneath the degenerated and ossifying cartilage. The primary spicules have been resorbed.  $\times 170$ .



3

Silberberg and Silberberg



4

Thyroxin and Estrogen

PLATE 199

FIG. 5. Growth zone at the upper end of the tibia of an untreated mouse of strain C57, 7½ months old. Growth of cartilage has ceased. The inactive cartilage still shows a faint columnar arrangement in addition to plugs of degeneration and ossification. Most primary spicules are resorbed.  $\times 170$ .

FIG. 6. Growth zone at the upper end of the tibia of a mouse of strain C57, 7½ months old, which had received 100 rat units of estrogen once weekly and 0.1 mg. of thyroxin three times weekly for 6 months. The epiphyseal plate still contains some inactive, heavily calcified cartilage, but most of it has been converted into a bony scar. Fragments of bony spicules are present.  $\times 170$ .

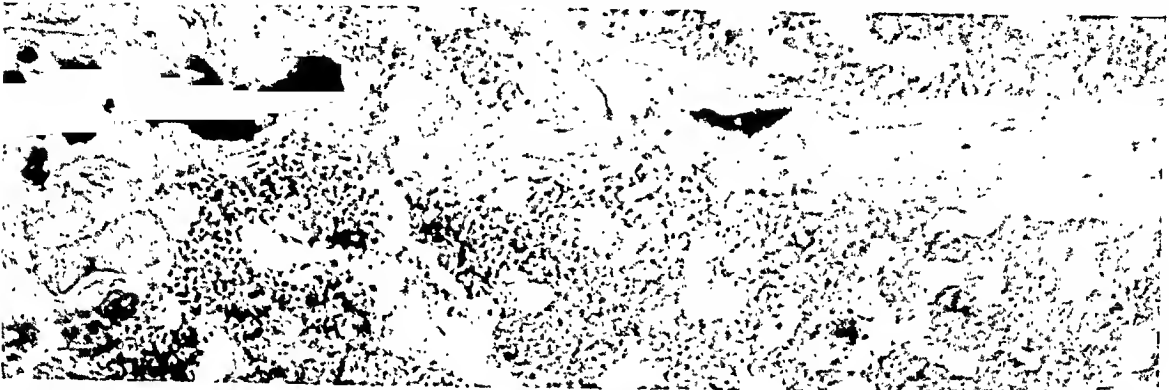
FIG. 7. Growth zone at the upper end of the tibia of a mouse of strain C57, 7½ months old, which had received 100 rat units of estrogen once a week for 6 months. The cartilage is heavily calcified, but shows little ossification and no tendency to perforate. The metaphysis contains a loose network of bony spicules.  $\times 170$ .

FIG. 8. Growth zone at the upper end of the tibia of a mouse of strain C57, 7½ months old, which had received 0.1 mg. of thyroxin three times weekly for 6 months. There is advanced epiphyseo-diaphyseal union, only thin osseous bars remaining of the former growth zone.  $\times 170$ .

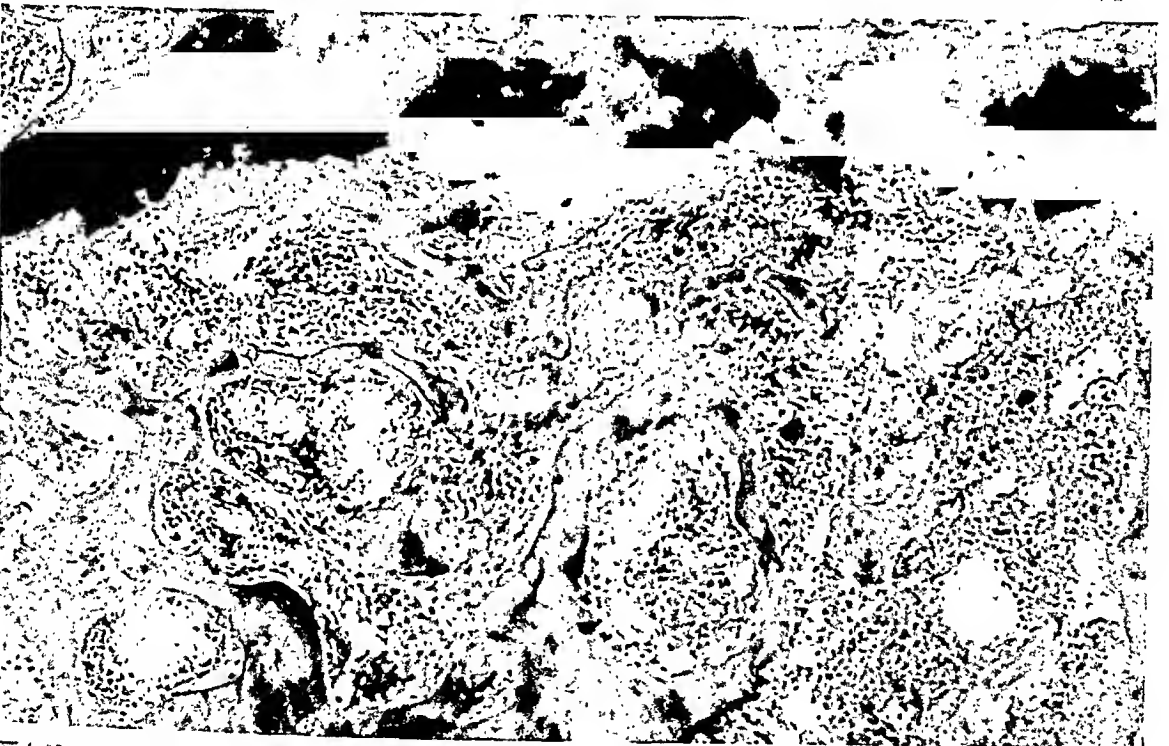
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6



7



8





# LEIOMYOMA OF THE VENTRAL LIGAMENT OF THE OVIDUCT OF THE CHICKEN \*

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During the course of studies on avian lymphomatosis at this Laboratory, several neoplasms other than those associated with the avian-leukosis complex<sup>1</sup> have been encountered. Of these, the leiomyoma has been most frequently observed, a finding which is in close agreement with that of other investigators. The myoma of smooth muscle has been identified in the chicken and described by several writers.<sup>2-9</sup>

This report will discuss the leiomyoma as it occurred in the 1943 hatched population of the breeding flock of pedigreed White Leghorns at this Laboratory. The apparent close association of the leiomyoma with the normal physiological process of egg production, together with its lack of interference with that function, suggest that the tumor described here will be of interest primarily to oncologists.

Both Jackson<sup>5</sup> and Goss<sup>6</sup> found leiomyoma to be the third most common tumor of chickens, being exceeded by leukotic tumors and adenocarcinomas. Olson and Bullis<sup>7</sup> found leiomyoma to be second in frequency to lymphocytoma. Goss found, in a survey of over 24,000 chickens, 20 cases of leiomyomas in a total of 1,445 birds which had neoplasms. Curtis<sup>8</sup> observed 8 tumors in the oviductal ligament in 880 birds, although she did not identify the tumors histologically.

This tumor had been identified in earlier populations at this Laboratory, but the incidence had been insignificant as compared with that in the 1943 hatched population.

The leiomyoma, according to Boyd<sup>10</sup> and Ewing,<sup>11</sup> is most frequently located in the reproductive organs, particularly in the uterus, although it may occur in other locations which contain involuntary muscle. Feldman<sup>4</sup> stated that in domestic animals the tumor may occur in the uterus, vagina, stomach, alimentary canal, or urinary bladder but is found also in association with the blood vessels, cervix, prostate gland, gallbladder, or spleen. According to Formad,<sup>12</sup> the tumor may also originate in the skin. The location in the chicken is similar to that in mammals in that the reproductive apparatus is the most frequent site.

Olson and Bullis<sup>7</sup> speculated on the causal relationship to egg production but stated that other factors must be involved since all hens with a record of prolonged, heavy production did not show increased incidence of leiomyoma.

\* Received for publication, October 2, 1945.

At this Laboratory attention was directed to a further investigation when 291 hens killed at 600 days of age revealed a leiomyoma incidence of 14.8 per cent. Of the 1943 breeding flock of 1,108 females, 59 birds showed the tumor at necropsy, a population incidence of 5.3 per cent. The detection of the tumor was incidental to necropsy of the birds that died during the course of other experiments, or were killed at the termination of the experiment. Since there was continuous mortality in this flock from various causes, only a part of the flock reached productive age. In 703 hens which had laid two or more eggs, the leiomyoma incidence was 8.4 per cent. This group included many birds which died prior to completing a year of production (at about 600 days of age) as well as many others that laid inconsistently. Restricting the data to hens which had completed a year of production, 43 of the 291 were positive for the tumor when examined at 600 days of age. All of these hens had produced eggs, and in all but 3 the organs were in a state of production at necropsy.

#### DESCRIPTION OF THE FLOCK

The 1943 flock of pedigreed Single Comb White Leghorns consisted of 15 lines.\* The birds of each line were the progeny of one sire and several dams, the latter being sisters or half-sisters. Thus within each line the hens under consideration were closely related. Each line consisted of from 4 to 8 families† with the exception of one line which contained 17 families. A total of 99 families had an average of slightly more than 11 females per family.

Except from one line, no apparently healthy birds were killed for necropsy prior to approximately 600 days of age. Mortality from lymphomatosis as well as from other causes occurred continuously, however, a factor which would tend to mask the propensity toward this tumor, particularly in those families which had a high mortality prior to heavy production. Two hundred and ninety-one hens were killed at 600 days of age, at which time they had completed 1 year of production.

#### DESCRIPTION OF THE TUMOR

*Gross Appearance.* Without exception the tumor was located in the ventral border of the ventral ligament of the oviduct about midway in its length. The ligament is enclosed by the peritoneum, the entire structure being referred to as the mesosalpinx. According to Curtis,<sup>14</sup> the thickened portion of the truncated apex contains smooth muscle fibers, some of which extend radially to, and encircle, the oviduct,

\* The term "line" refers to a group of birds with a relatively high *inter se* relationship between sibs or near relatives resulting from restricted ancestry.<sup>13</sup>

† "Family" refers to the progeny of one male and one female.

anastomosing with the muscle fibers of the wall. The tumor developed at the point of convergence of the blood vessels in the mesosalpinx which supply the oviduct. The various other locations described in the literature were not found to be tumor sites in the examples identified.

The 59 leiomyomas of this population ranged from 3 to 40 mm. in diameter. They were irregularly spherical, or were somewhat elongated, particularly those of small dimensions. The tumors were pink to white and some of the larger specimens were marked by large convoluted vessels below the capsule. There was little blood present in the body of the neoplasm, probably due to the density of the structure. The capsule was firm and everted only slightly on section. The tumor itself was firm, dense, and resistant to incision. In the small tumors the consistency was firm but doughy under pressure. The cut surface showed fibrous appearing septa, irregularly placed. These areas had the characteristic sheen of fibrous tissue.

The tumor appeared to have developed symmetrically from the thickest part of the ligament. There was no peduncle formation. The gross appearance was that of a well encapsulated, compact tumor (Fig. 1). It had not reached sufficient size in any case to have interfered mechanically with the passage of the egg through the oviduct, nor with egg expulsion. There was no evidence of metastasis.

*Microscopical Appearance.* Specimens from 4 cases were fixed in Petrunkevitch's cupric paranitrophenol<sup>15</sup> and sections were stained with hematoxylin and triosin or by Masson's connective tissue method. Three of the cases were considered representative specimens and had shown gross characteristics typical of the majority. These tumors were 4, 7, and 34 mm. in diameter and in each case the hen was in egg production. The fourth case was from a hen in which the tumor appeared to be undergoing regression.

In general, the histological picture was similar to that described by Ewing<sup>11</sup> and Olson and Bullis.<sup>7</sup> There were interlacing bundles of smooth muscle fibers which ran in various directions, giving a whorled appearance to the whole (Fig. 2). The specimens varied in the amount of stromal connective tissue present. The bundles were separated by fibrous tissue and intercellular fibrils were present. There was no indication of inflammation but some round cells were present in 2 cases. In addition to the main bundles of smooth muscle, there was proliferation of the muscle layer of the vessel walls.

In those tumors which appeared to be undergoing regression, there was a greater proportion of connective tissue, not only in the septa but throughout the areas of muscle tissue.



## INCIDENCE AND RELATIONS

*Lines.* The incidence of leiomyoma in the entire population, in hens that had laid, and in those that lived for 600 days, has been given in the introduction. The leiomyoma was present in 10 of the 15 lines of chickens and was not identified in 5 lines. In the 10 lines carrying the neoplasm, the incidence ranged from about 1.5 to 18 per cent. However, when only hens that had laid are considered, the incidence ranged from 2.0 to 27.3 per cent. When the number of hens was further restricted to those that had lived to 600 days (all of which had laid), the incidence was from 3.4 to 50 per cent (Table I). Five lines had

TABLE I

*Incidence of Leiomyoma in 15 Lines Figured on Total Flock, Females That Laid, and Females That Lived to 600 Days*

Line	No. of cases of leiomyoma	Total no. of ♀♀	Tumor incidence	Total no. of ♀♀ that laid	Tumor incidence	No. of cases of leiomyoma	No. of ♀♀ that lived to 600 days	Tumor incidence
			(per cent)		(per cent)			(per cent)
1	3	48	6.25	30	10.00	2	16	12.50
2	0	53	0.00	21	0.00	0	7	0.00
3	9	50	18.00	33	27.27	9	18	50.00
4	2	61	3.28	42	4.76	2	21	9.52
5	0	47	0.00	25	0.00	0	19	0.00
6	1	68	1.47	49	2.04	1	29	3.44
7	1	75	1.33	50	2.00	1	29	3.44
8	0	57	0.00	36	0.00	0	13	0.00
9	5	52	9.62	25	20.00	3	10	30.00
10	4	95	4.21	56	7.14	3	39	7.69
11	0	40	0.00	14	0.00	0	2	0.00
12	1	51	1.96	42	2.38	1	14	7.14
13	11	72	15.30	50	22.00	11	31	35.48
14	0	80	0.00	50	0.00	0	11	0.00
15	22	259	8.49	180	12.22	10	32	32.25
Total	59	1,108	5.32	703	8.39	43	291	14.77

no leiomyomas; 5 lines had an incidence of from 2 to 5 per cent, and 3 lines an incidence of about 6 to 10 per cent. The remaining 2 lines ranged from about 15 to 18 per cent. These data suggest that this tumor was more prevalent in hens which had laid over a prolonged period and that there was a marked variation in incidence between lines.

*Families.* The tumor was present at necropsy in 30 of 99 families. Each of the 10 positive lines contained some families which were negative for this tumor. In other words, in no line was the tumor present in all families of that line. It might appear, *a priori*, from the incidence in certain lines and families, that genetic susceptibility plays the most important part in the occurrence of the leiomyoma. However, genetic influence was apparently masked by other disposing factors of age and egg production as well as the intercurrent disease of lympho-

matosis. Lines and families which were susceptible to lymphomatosis died at an age below that of the heavy production period. Under such circumstances the propensity to this neoplasm would not become manifest.

*Age.* Birds of the 10 positive lines averaged 415 days of age and those of the 5 lines which were negative averaged 333 days of age at necropsy. All negative lines were lower in average age at necropsy than the average of the positive lines; however, of the 10 positive lines, one line was below the negative line average. The absence of the tumor in the lines which died at an earlier average age suggests that the tumor might have occurred within those lines had the life span equalled that of the positive lines. Disregarding lines or families, birds which showed no evidence of the neoplasm averaged 371 days of age at necropsy,

TABLE II

*Number of Hens without Tumors Which Laid More Eggs than Average of Line*

Positive lines	Average number of eggs by positive ♀♀	No. of negative ♀♀ which laid more than average of positive ♀♀
1	188	2
3	213	2
4	166	12
6	127	23
7	301	3
9	228	3
10	237	2
12	167	12
13	206	9
15	215	7

while positive birds averaged 591 days of age when examined. There were, however, 236 negative laying hens which were older than 591 days at necropsy. Likewise, there was one hen with leiomyoma under 371 days of age at necropsy. These data illustrate the overlapping of ages in the positive and negative birds, and indicate that age alone is not the most important factor in the occurrence of leiomyoma.

*Egg Production.* The positive birds which completed a year of production averaged 199 eggs for that year; whereas, laying negative birds averaged 155 eggs for a similar period. In the entire population there were 46 negative hens, 600 days old, which laid more eggs than the average of the positive hens. The production for hens which completed the first year of laying ranged in the positive cases from 126 to 250 eggs while negative laying hens for a similar period ranged from 2 to 281 eggs. All 10 positive lines contained some negative hens which laid more eggs than the average of the positive cases in that line (Table II).

*Tumor Size.* There was no definite relation between tumor size and the number of eggs laid during a given period. Fifteen hens with tumors 3 to 5 mm. in diameter averaged 195 eggs for the first year of laying and 6 hens with tumors of from 20 to 40 mm. in size averaged 215 eggs for the first year of production. Including hens which did not complete a full year of laying, 22 birds with tumors 3 to 5 mm. in diameter averaged 15 eggs per month for the 6 months prior to necropsy, exactly as did 11 hens which had tumors that were 20 to 40 mm. in diameter. In positive birds which had laid no eggs 30 days prior to necropsy the neoplasm averaged 7.9 mm. in diameter. In positive hens which laid 1 to 10 eggs for this period the tumor averaged 13.1 mm. in size, and in hens which laid more than 20 eggs during the 30 days preceding necropsy the neoplasm averaged 10.8 mm. in diameter. Tumor size plotted against the number of eggs laid for 1, 2, 3, or 6 months prior to necropsy showed no definite correlation. In 4 of the 59 cases showing the leiomyoma, the ovary was not in a laying state at necropsy. One hen (E802D) had laid 194 eggs in the first year of production but had laid no eggs for 6 weeks prior to necropsy. The tumor in this case was 4 mm. in diameter. A second hen (E813K) produced 163 eggs the first 12 months of laying and laid 11 eggs in September. She was killed on October 10th and was found to have a tumor of the mesosalpinx, 8 mm. in diameter. Another hen (E257D) had laid continuously for the 10 months before necropsy, producing a total of 175 eggs for that period. This hen had laid 3 eggs in October and was killed October 10th. The neoplasm was 40 mm. in diameter. The fourth bird (E764W) had a 4 mm. tumor. This hen had laid 167 eggs for the first year of production but no eggs were recorded for her for 3 months prior to necropsy. For 2 hens (E606A and E615X) the record showed that no eggs were laid for 7 and 4 months respectively before necropsy. However, at necropsy the ovaries were either in a state of production or approaching production. It should be stated, also, that there was one hen with tumor as well as several negative birds for which there were no records of eggs. It is probable that these hens were "floor-layers."

#### DISCUSSION

The incidence of leiomyoma in the 1943 hatched flock at this Laboratory was much higher than has been previously reported.<sup>5-7</sup> The incidence in this flock was likewise much higher (nearly 15 per cent) in the group of 291 birds which were killed at approximately 600 days than in birds (about 2 per cent) which were necropsied at ages below 600 days. However, the latter group contained one line of birds which

were destroyed at ages between 500 to 600 days. In the 1,108 birds, only 5 of the 59 neoplasms were detected in birds that had died; whereas, 54 cases were identified in hens that were killed. Of the negative birds which had died, the ovary was found only infrequently to be in a state of production. In such cases cannibalism was usually the cause of death. In the 5 hens which died and which showed the neoplasm at necropsy, the ovary was either in a state of production or a state of regression from recent laying.

It is probably true that a flock of 300 laying hens, as described here, is seldom available for necropsy examination. The high incidence of leiomyoma in this cross section at 600 days suggests that the tumor must tend to regress after the cessation of laying.

Since the tumor occurs with greater frequency in laying birds and apparently regresses, its detection would ordinarily be largely limited to such birds. The tendency to submit only morbid or obviously diseased birds for necropsy would thus result in a lower necropsy incidence than when laying birds are included.

The fact that the tumor did not appear in 5 lines suggests that a hereditary influence is involved. All of the 5 negative lines contained some individuals which had production records equal to those of the positive birds. In general, however, the negative lines and families died at an earlier average age than did the lines or families in which the neoplasm was present. The families that died at an early age—below that necessary for heavy production—obviously did not show the neoplasm. However, an average age of death for a family equal or over that of positive families did not necessarily mean that the neoplasm would be present. In some families which had only one member with neoplasm, the age and production records of that bird were higher than the family average. The fact that the neoplasm was not found in 5 lines suggests that some genetic influence operates to predispose birds of certain breeding to the tumor, provided the egg production factor is also considered.

The age at which the tumor is most likely to appear could not be determined from the data available although from the foregoing it is apparent that the hen must reach maturity since sustained production was a closely associated factor.

The tumor was found only in hens that had produced eggs and usually in the best layers. One hen, however (E1101J<sub>3</sub>), laid only 40 eggs over a period of 11 months. This hen had laid from 1 to 5 eggs per month. In this case the leiomyoma was only 5 mm. in diameter. Curtis<sup>8</sup> described the ventral border of the ventral ligament as a "muscular cord," 3 to 6 mm. in diameter. That description would

suggest that such a size should be considered normal. In this report such a size was considered as gross evidence of tumor involvement. The one 4 mm. specimen examined microscopically revealed proliferation of the muscle layer of the vessel walls.

The fact that some hens without tumors had an egg record equal to that of those with tumors within each line suggests that there is, besides heredity and egg production, a third etiologic factor.

There was no relation between tumor size and the number of eggs laid during a given period. However, since neither the time of onset of the neoplasm nor its rate of growth is definitely known, the data given are of questionable value. The fact that the hens with the larger tumors laid only 20 eggs more over a 12-month period than did those with the smaller tumors would suggest that such a slight increase in production would hardly account for the great difference in tumor sizes.

In the flock examined the incidence of this neoplasm was second only to lymphomatosis, which is in agreement with the report by Olson and Bullis.<sup>7</sup> However, in birds killed at 600 days the incidence of leiomyoma was higher than that of lymphomatosis.

The ventral ligament of the oviduct was the only site of leiomyoma in the flock under discussion. This would indicate that the neoplasm is closely associated with the reproductive processes. Other writers have described this neoplasm in several locations in the same bird and have suggested the possibility of metastasis. Jackson<sup>5</sup> stated that all tumors of the smooth muscle cannot be regarded as true leiomyomas, since implantation metastasis from a carcinoma leiomyomatosum may take place with subsequent regression of the epithelial elements in the implant.

Since the neoplasm was observed in only one location in the group examined, that fact as well as the density, encapsulation, and the apparent slow growth of leiomyomas would tend to favor the theory of multiple origin rather than of metastasis when the neoplasm is found in more than one location in the body.

The probability that the tumor is transient, coupled with the fact that hens are frequently not laying when in a morbid condition, paradoxically associates the neoplasm with only laying birds or birds which are normal aside from the tumor. This relationship with normal physiologic activity is unique, especially since the neoplasm does not appear to interfere with the function of laying. Such an association naturally raises the question of whether the leiomyoma is a true neoplasm.

That hypertrophy of the smooth muscle of the ovarian ligament is not necessary for high production is shown by the fact that the de-

velopment referred to as a neoplasm in this report is not consistently found in all high producers. Prolonged functioning of the reproductive organs might conceivably stimulate the unrestricted proliferation of smooth muscle tissue. The pull on the apex of the ligament might thus cause hyperplasia of the smooth muscle fibers. It was noted grossly that the subcapsular vessels were often engorged and convoluted, indicating a local high blood pressure. Such pressure might conceivably be the result of interference with circulation by the contraction of the fibers in the main tumor. If such a process does occur, the increased intravascular pressure might account for the thickening of the vessel walls in that region. Acceptance of this explanation would result in classifying this tumor as a hyperplastic rather than a neoplastic process. Mechanical functional stimulation from continuous production would be indirectly related to a high level of the female sex hormone.

The fact that the tumor does not occur in all birds of equal production records suggests that some unidentified factor is operating to stimulate the muscle tissue to proliferate. In contrast to the above theory of functional stimulation, there may be a direct relationship with the blood level of the female sex hormone. The increase in size of the reproductive organs to accommodate pregnancy in the mammal has its counterpart in the fowl in the hypertrophy of structures associated with egg production. The female sex hormone undoubtedly plays a part in the functional development of the oviduct for egg production and in egg production itself. An extension of the physiological hypertrophy due to a continuous high level of the hormone in the blood is thus conceivable.

While a genetic influence was not definitely identified, the distribution of the occurrence of the tumor suggests that some hereditary relationship does exist.

#### SUMMARY

1. The leiomyoma of the ventral ligament of the oviduct is of oncological interest because of its close association with normal physiological processes.
2. The relatively high incidence (14.8 per cent) in laying hens killed at 600 days suggests that the tumor may be more common than has been reported heretofore.
3. The gross and histological features indicate that the tumor is benign.
4. The tumor does not appear to interfere with egg production.
5. Theories on the factors related to causation are suggested: (a)

mechanical functional stimulation, (b) increase in the level of the female sex hormone.

6. Since the tumor is not known to be malignant nor to interfere with normal functions, it should be of little concern to the poultry breeder.

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#### DESCRIPTION OF PLATE

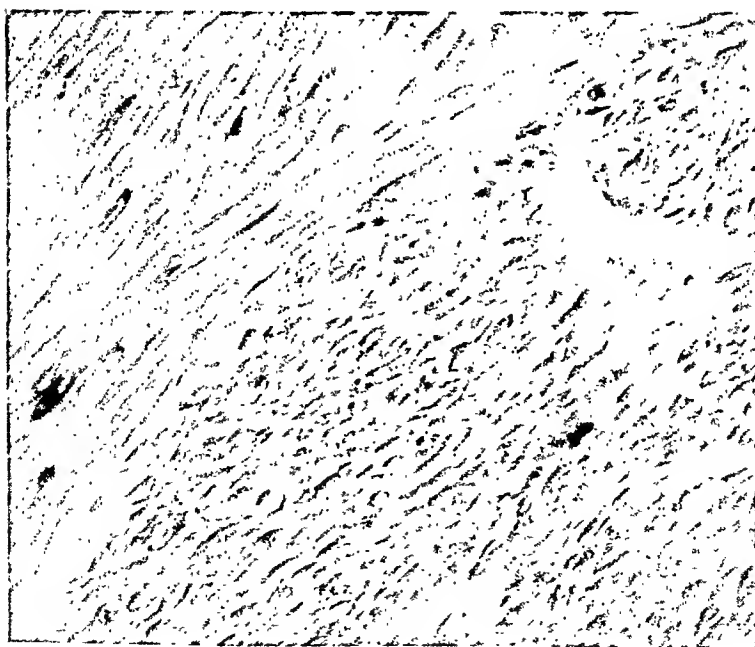
##### PLATE 200

FIG. 1. Leiomyoma of the ventral ligament of the oviduct of the hen.  $\times \frac{1}{2}$ .

FIG. 2. The histological structure and whorled appearance of the tumor of the ventral oviductal ligament are typical of leiomyoma.  $\times 300$ .



1



2





# THE OCCURRENCE OF NEOPLASMS IN THE LIVER, LUNGS, AND OTHER TISSUES OF RATS AS A RESULT OF PROLONGED CHOLINE DEFICIENCY \*

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The influence of diet and separate dietary constituents on the incidence of neoplasms induced by carcinogenic chemicals has been studied by various investigators with conflicting results. So far as we are aware, however, the production of neoplasms as a result of a simple dietary deficiency has not been reported.

Hepatic cirrhosis in animals receiving choline-deficient diets has been produced by several groups of workers.<sup>1-4</sup> An experimental procedure for the consistent production of such a cirrhosis was developed in this laboratory and an intensive study of the pathology involved was undertaken. In the course of the study, the development of various types of neoplasms was observed in what appears to be a significant percentage of the experimental animals. It is the purpose of this paper to describe the sequence of lesions culminating in the appearance of these neoplasms in the liver and other tissues of rats receiving a choline-deficient diet.

## EXPERIMENTAL PROCEDURE

In a series of feeding experiments on different diets, it was found that Diet 43A was suitable for producing the lesions described in this paper. Diet 43A consists of 30 per cent alcohol-extracted peanut meal, 6 per cent alcohol-extracted casein, 40 per cent sucrose, 20 per cent pure lard, and 4 per cent salt mixture.<sup>†</sup> The peanut meal was extracted five times and commercial casein was extracted four times in boiling 95 per cent ethyl alcohol for 2-hour periods. The peanut meal was the usual commercial oil mill product containing 45 per cent of protein. To each kg. of the diet were added 200 mg. of inositol, 10 mg. of calcium pantothenate, 50 mg. of alpha-tocopherol, 4 mg. of riboflavin, 2 mg. of thiamine, 4 mg. of carotene, 2 mg. of pyridoxine, and 0.125 mg. of calciferol.

Rats were started on the experimental diet at the age of 23 days, in individual cages with raised screen bottoms. They received Diet 43A

\* Received for publication, October 15, 1945.

Published with the approval of the Director of the Alabama Agricultural Experiment Station.

<sup>†</sup> Salt mixture 186 modified by the addition of Cu, Zn, and Mn was used. (Guerrant, N. B., and Salmon, W. D. The stability of vitamin G as measured by its growth-stimulating effect. *J. Biol. Chem.*, 1930, 89, 199-211.)

*ad libitum*. Two rats from each litter were designated as controls and were fed 20 mg. of choline chloride a day for the duration of the experiment. A total of 88 rats were placed on Diet 43A; of this number, 19 were controls receiving choline throughout the experiment. In order to prevent death from acute choline deficiency, it was necessary to feed predetermined minimum supplements of choline to the experimental rats for the first 2 weeks. After this, the dosage was gradually decreased until the experimental animals were deprived of choline entirely. In case of serious weight loss soon after the choline was discontinued, an occasional dose was administered. The aim was to use the minimum dosage necessary to keep the rats alive.

The rats were weighed each day during the first 6 weeks and once a week thereafter. Some were killed for necropsy at regular intervals and their organs preserved for histopathologic studies, so that the sequence of changes occurring in the tissues could be followed; others were kept on the experimental diet until marked symptoms of decline in condition were evident. The animals were killed by decapitation. At necropsy, tissue specimens were taken from each lobe of the liver, and from the spleen, pancreas, lymph glands, lung, kidney, adrenal, thymus, heart, alimentary tract, and any abnormal growth. The tissues were fixed in Bouin's, Zenker-formol, Zenker-acetic, formol, or 70 per cent ethyl alcohol fixatives. Both frozen and paraffin sections were made. The following staining technics were used: hematoxylin and eosin, scarlet red and hematoxylin, Taenzer-Unna orcein and methylene blue, osmic acid, Mallory's triple stain, Nile blue sulfate, Weigert's stain, and Masson's trichrome stain for connective tissue.

## RESULTS

### *Symptomatology*

Fourteen (20 per cent) of the rats not receiving choline died of acute choline deficiency during the first 6 weeks on the experimental diet. The remainder of the choline-deficient rats made a moderate growth and remained in reasonably good condition for several months. Following this they gradually became unthrifty in appearance. There were marked loss of hair, muscular weakness, drowsiness, and lethargy. In some, the eyes became lusterless and opaque following earlier hemorrhage. Râles were common in the later stages of the condition.

### *Early and Transitional Lesions*

In animals necropsied during the first 28 days of feeding on the choline-deficient diet, the livers were swollen, abnormally light in color, and increased in weight above the normal. There was a marked ac-

cumulation of fat in the form of globules of various sizes, apparently involving all the liver cells. Some cells were so filled with fat globules that the nucleus was pushed to one side. The staining reactions indicated the presence of both fatty acids and neutral fats.

Necrosis occurred as a sequel to earlier phases of cell degeneration. The first indications of necrosis were distention of the nucleus followed by its shrinkage and condensation into several deeply staining fragments.

Sections from livers of rats that had been on the choline-deficient diet for 3 months showed the peculiar zonal distribution of fatty infiltration and degeneration illustrated in Figure 1. This was designated as a pre-cirrhotic or mild cirrhotic stage. In the fatty zones considerable necrosis was noted and there was a definite increase in fibrous tissue in these regions. In some lobes of the liver, the fibrous tissue completely encircled lobules and groups of lobules; there was also considerable increase in the intralobular connective tissue. Evidences of a rapid rate of cell destruction and cell regeneration began to appear.

#### *Advanced Lesions*

All rats that received the choline-deficient diet for 8 months or longer developed advanced hepatic cirrhosis. There was considerable variation in the gross appearance of these livers; some were almost smooth on the surface; others were only slightly roughened or finely granular; the most severe had the characteristic "hobnail" appearance with nodules and pedunculated projections varying from the size of a pin-head to 1 cm. in diameter (Fig. 2). The consistency varied from lobe to lobe. In general, the livers which were least severely affected were largest and lightest in color, indicating the highest fat content. Of the livers examined in this period, 85 per cent were classified as "hobnail" cirrhosis. The typical microscopic appearance of these livers is illustrated in Figure 3. Most of them showed the characteristic perilobular fibrosis with intralobular strands of connective tissue. In most cases the intralobular strands were so prominent as to cause a pseudo-lobulation.

There was considerable variation in the amount of fat in the different lobules of the liver, the fat appearing to decrease as the cirrhosis advanced in extent and severity. While some of the lobules contained considerable fat, others consisted largely of newly regenerated liver cells and contained little or no fat; some were in a state of partial necrosis. This variation is shown in Figure 4.

In 75 per cent of the cirrhotic livers there was a substance distributed in globular form throughout the interlobular connective tissue, and in some cases in the lobules themselves (Fig. 5), that was not dis-

solved by alcohol, dioxane, or xylol. This substance stained red with sudan IV, blue to blue-black with Nile blue sulfate, and black with osmic acid. It is probably identical with the ceroid of Lillie and co-workers,<sup>5</sup> although Diet 43A did not contain cod-liver oil. Its distribution indicated that it is a product of cell degeneration.

The livers of 50 per cent of the animals on the choline-deficient diet contained cysts, which appeared to be agglomerates of dilated sinusoids interrupting the regular architecture of the lobule. There was a focal loss of parenchymal cells, with a proliferation of endothelial cells in these areas and a considerable amount of collagenous fibers between the cyst-like spaces. The cystic spaces in any one area varied in size as shown in Figure 5. These spaces contained a small amount of fibrinopurulent exudate. The cells lining the spaces were in most cases endothelium-like, indicating that they might be derived from the sinusoids. In some cases, however, the spaces were lined by cuboidal or even low columnar cells, indicating that they might be derived from bile ducts. In some lobes of the livers, cells of the endothelial type were so predominant that the regular parenchyma was obscured. The proliferation of endothelial cells with a focal loss of parenchymal cells is illustrated in Figure 6. Areas of this type were very numerous and were found consistently in all the cirrhotic livers.

In some lobes of the livers very numerous mitotic figures were found, indicating that regeneration of new parenchymal cells was taking place at a rapid rate. The portal veins in some of the livers were altered. Their walls were thickened in places by proliferation of connective tissue and the deposition of a substance staining deep blue with hematoxylin. In the most severe cases of cirrhosis of the liver, severe hyperplasia was evident in the lobules and pseudo-lobules as shown in Figure 7. In some lobules there were numerous giant cells arranged in acini and cords resembling a hepatoma (Fig. 8).

In 10 per cent of the animals, there were pedunculated projections from the liver which were larger than the hyperplastic nodules previously described. These neoplasms were usually multiple, varied in size, and appeared to be hepatomas. Some were connected to the liver only by a thin strand of connective tissue, but others were in the mesentery entirely separated from the liver. Some were found in the midst of the hyperplastic liver parenchyma, compressing this tissue. Figure 9 illustrates the distribution of these neoplasms. Histologic preparations of these nodules showed no tendency toward lobulation, a peculiar cell arrangement, and very numerous mitotic figures (Fig. 10). There was very little connective tissue in these areas. As shown in Figure 11, the cytoplasmic membranes were very prominent. The cells varied

in size and, in some parts of the growth, they were arranged in solid sheets, while in others they were arranged in whorls and acini-like structures or in cords of varying thickness (Fig. 12). Some of the cells showed atypical tricentric mitosis. A large percentage of the cells contained from two to four nuclei, and the nucleoli were unusually prominent. Some nuclei were oblong in shape or extremely large and contorted, having one or more indentations. The staining reaction was unlike that of normal liver cells and these areas could be distinguished in the slide because of this difference.

Extensive bile duct proliferation was found in all of the livers of the experimental animals. In most of them these proliferations seemed to follow the connective tissue bands, but in some the growth was so extensive as to involve large numbers of entire lobules and to replace as much as one-third of an entire lobe of the liver. All lobes of the liver were sometimes affected.

The livers of 30 per cent of the 50 rats that survived for 8 months or longer on the choline-deficient diet exhibited neoplasms of the nature of an adenocarcinoma. This growth was entirely different from the numerous areas of proliferating bile ducts; it was characteristically made up of cords of cells, solid masses, and acini-like whorls of stratified cells. The acini-like spaces contained cell debris and fibrinous exudate. The growth differed from the bile duct proliferations in that the epithelial cells of which it was composed were definitely stratified and not in simple acini. There were large numbers of mitotic figures present and there seemed to be little relation between the epithelial cells and the stroma. This type of lesion was not necessarily located at the periphery of the liver and was not represented by a shrunken area, but rather by a massive growth at times compressing liver tissue. As to the origin of the growth, it seemed that in some instances it arose from the liver cells themselves, as can be seen in Figure 13. In some the growth began at the center of the lobule, and in others it seemed to arise in the interlobular spaces, as shown in Figure 14. In still other examples the growth seemed to arise from the cells on the outer margin of the lobule. The growth was so extensive in 25 per cent of the cases that one-third of the lobules of an entire lobe were affected and more than one lobe of the liver was concerned. Figure 15 illustrates the pattern of the growth.

Hemangio-endotheliomas in the peritoneal and subcutaneous fat were found in 10 per cent of the deficient animals. Three of the animals exhibited subcutaneous growths measuring from 1 to 5 cm. in diameter and located in the inguinal region. Two of the animals had growths that were smaller but of the same type located in the peritoneal

fat. Microscopic examination revealed extremely cellular hemangio-endotheliomas with large numbers of blood vessels and vascular spaces filled with blood cells. In some cases the structure resembled that of erectile tissue. The cells contained oval or round nuclei with scant chromatin. The cytoplasm was indistinct and stellate or streaming in appearance. Very many mitotic figures were seen in two of the growths but the rest showed only a moderate number. Figure 16 illustrates the structure of this growth.

The lungs of 38 per cent of the animals necropsied after 8 to 10 months on the choline-deficient diet showed nodules varying in size and number, involving various parts and in some cases practically all of the lung. One of the more severely involved lungs is pictured in Figure 17. As a routine procedure, smears were made of the fresh material from these nodules and fixed and stained according to the Ziehl-Neelsen technic. This test showed no acid-fast organisms. Likewise, cultures prepared on various media failed to demonstrate acid-fast organisms. Material from freshly dissected rats was prepared and injected into guinea-pigs. The sites of injection healed without the development of ulcers. After the animals remained healthy for several months they were killed for necropsy. The lymph glands were normal grossly and histologically, as were also the other organs of the body.\*

Sections of the pulmonary nodules revealed an abnormal growth consisting of masses of anaplastic oval cells of various sizes. There were numerous acini-like structures of an adenomatous nature, but the larger masses of cells were of the nature of a primary, medullary carcinoma. Figure 18 illustrates the oval or spindle-shaped cells with very prominent oval or round nuclei that stained heavily with hematoxylin. Mitotic figures were very numerous.

The epithelium lining bronchiectatic cavities exhibited marked metaplasia in a number of cases. Figure 19 illustrates a transition of the simple columnar epithelium lining a bronchiectatic cavity to a well differentiated stratified squamous epithelium. Numerous bronchiectatic cavities were present, lined with hyperplastic epithelium which exhibited many mitotic divisions; in most cases there were papillomatous invaginations of epithelial character into these cavities. The papillomatous structures contained sheets of cells which had prominent oval nuclei. The cytoplasmic borders of these cells were ill defined (Fig. 20).

\* We wish to express our appreciation to Dr. W. E. Cotton of the School of Veterinary Medicine for assistance in making the tests for acid-fast organisms and the guinea-pig inoculations.

Of the 50 animals, 3 developed retroperitoneal growths, which, through pressure on nerves, produced paralysis of the posterior extremities. The largest of these growths was 3 by 2 by 1 cm., and the smallest was 1 cm. in diameter and rounded. The masses were of firm consistency and were reddish on gross dissection. Microscopic examination revealed neoplasms of the nature of a sarcoma or fibrosarcoma. The amount of connective tissue varied in the different parts of the growths; in some places it was very prominent, but in other parts of the growth it was almost imperceptible or entirely absent. The most characteristic sections of this growth, as shown in Figure 21, were composed of spindle-shaped cells of moderate size, arranged in rows in different planes and closely packed together. In some parts of the growth, connective tissue was more prominent (Fig. 22) but the arrangement of the cells was still characteristically the same. In some places there was infiltration of the muscle layer with destruction and replacement of the normal tissue (Fig. 23). In all of the neoplastic tissue there were very numerous mitotic figures, and in some cases abnormal divisions. Figure 24, which is enlarged to show the nature of the spindle-shaped cells and their nuclei, includes one of these abnormal division figures.

#### DISCUSSION

The occurrence of neoplasms of various types in 58 per cent of the rats that were kept on a choline-deficient diet for 8 months or longer, while not a single neoplasm developed in litter-mate controls receiving the same diet supplemented with 20 mg. of choline per rat daily, emphasizes the apparent significance of the results.

It appears that marked accumulation of fat in the liver cells resulting from an insufficiency of choline, or more specifically of labile methyl groups, initiates a sequence of responses which result in profound changes in the structure and behavior of the cells. Cell degeneration and cell regeneration are greatly stimulated; connective tissue cells replace normal liver cells; extensive bile duct proliferation, severe hyperplasia, and exaggerated mitotic activity occur; and eventually the abnormal types and patterns of the neoplastic cells appear. The sequence of changes is remarkably similar to that reported in the literature following the administration of carcinogenic hydrocarbons. Interestingly enough, some of the diets used in the study of carcinogenic compounds<sup>6, 7</sup> have been low in methionine and choline. White and Edwards<sup>8</sup> have reported, however, that supplements of methionine and choline do not decrease the incidence of p-dimethylaminoazobenzene-



induced hepatic tumors. On the other hand, Maisin and co-workers, Nakahara, Mori, and Fujiwara, and other investigators are reported by Cook and Kennaway<sup>9</sup> to have shown the presence of anticarcinogenic factors in brain, liver, thymus, bone marrow, yeast, and whole rye flour.

The sequence of changes in the lungs was not followed as closely as in the liver. As previously reported from this laboratory,<sup>10</sup> severe congestion, hemorrhage, and edema in the lung appear in the early stages of choline deficiency. Hydrothorax is a common finding. These early lesions probably are the basis for the development of primary carcinoma in a relatively high percentage of the animals.

### SUMMARY

A chronic or prolonged choline deficiency was produced in rats by feeding a diet low in choline and methionine but supplemented by minimum doses of choline to prevent death from acute choline deficiency. Of the 88 rats started, including the controls, 50 survived for 8 to 16 months on the choline-deficient diet. The pathology of chronic choline deficiency in these animals was studied.

Cirrhosis of the liver was consistently found in all of the rats on the deficient diet. A substance resembling "ceroid" was observed in 75 per cent of the cirrhotic livers.

Cysts, characterized by altered sinusoids and bile ducts with a focal loss of parenchymal cells and a proliferation of endothelial cells, were found in the livers of 50 per cent of the animals.

Hepatoma-like neoplasms developed in 10 per cent of the livers. These neoplasms were also found in the mesentery, with a thread-like attachment to the liver in some cases and without any apparent attachment to the liver in others.

Neoplasms of the nature of adenocarcinoma were observed in the livers of 30 per cent of the animals.

Neoplasms that appeared to be primary carcinomas occurred in the lungs of 38 per cent of the rats.

Subcutaneous and peritoneal hemangio-endotheliomas were found in 10 per cent of the animals.

In 6 per cent of the rats, neoplasms that were classified as retroperitoneal sarcomas were found.

Neoplasms of one or more types were observed in 58 per cent of the deficient animals.

No lesions similar to those described were found in the litter-mate controls, receiving the same diet supplemented with 20 mg. of choline chloride per rat daily.

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[ Illustrations follow ]

## DESCRIPTION OF PLATES

### PLATE 201

- FIG. 1. Zonal distribution of fat and some cellular degeneration and fibrosis in the liver of a rat after 90 days on choline-deficient Diet 43A. Hematoxylin and eosin stain.  $\times 120$ .
- FIG. 2. Variation in the gross appearance of cirrhotic livers from different rats on Diet 43A.
- FIG. 3. Extensive perilobular fibrosis and the alteration of sinusoids in the cirrhotic liver of a rat after 8 months on Diet 43A. Hematoxylin and eosin stain.  $\times 145$ .
- FIG. 4. Variation in cell construction and fat content of different lobules of a cirrhotic liver. Hematoxylin and eosin stain.  $\times 300$ .

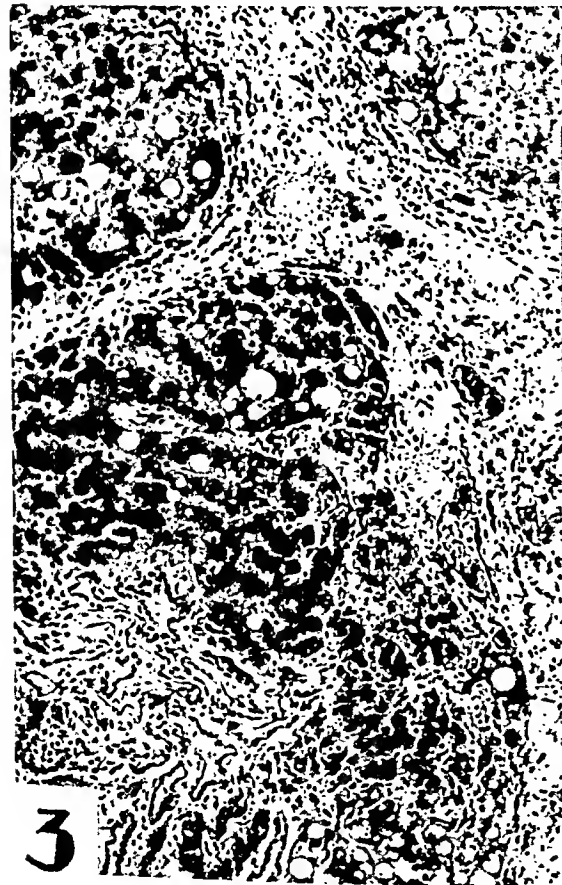
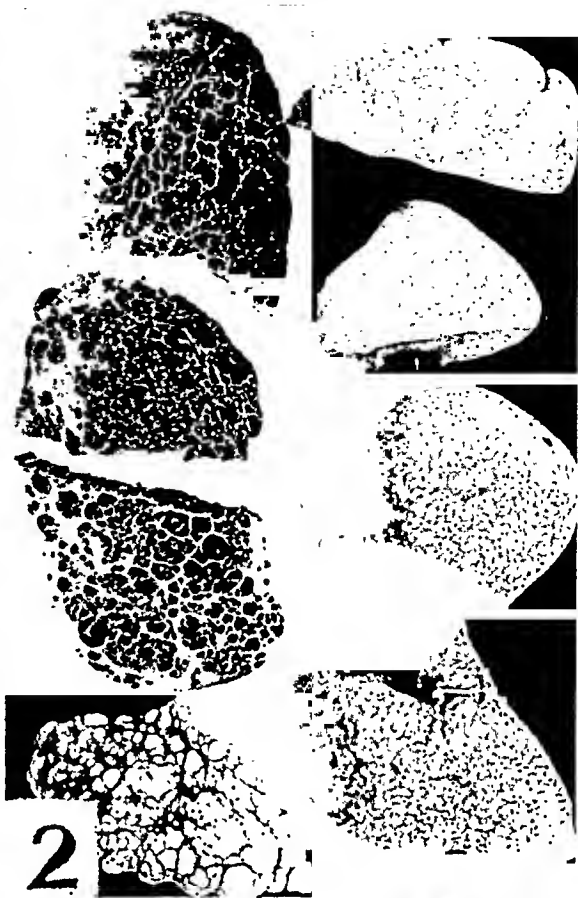
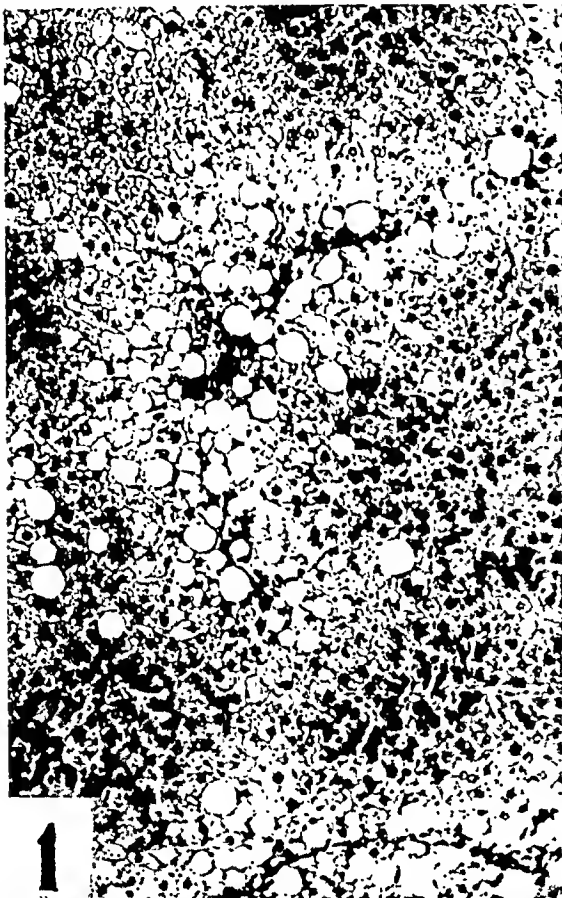


PLATE 202

- FIG. 5. Liver of a rat after 10 months on Diet 43A, exhibiting cirrhosis, deposition of an insoluble pigment, and cysts lined by flattened endothelium-like cells. There is a focal loss of parenchymal cells and a proliferation of spindle-shaped cells. Hematoxylin and eosin stain.  $\times 130$ .
- FIG. 6. Area of the liver of a rat showing focal loss of parenchymal cells and a proliferation of endothelium-like cells. Hematoxylin and eosin stain.  $\times 300$ .
- FIG. 7. Hyperplastic nodule on a cirrhotic liver showing a significant increase in parenchymal cells, small in size and peculiar in arrangement. Hematoxylin and eosin stain.  $\times 400$ .
- FIG. 8. Area of liver showing giant cells with a peculiar staining reaction, granular cytoplasm, and a cord-like arrangement of some of the cells. Hematoxylin and eosin stain.  $\times 300$ .

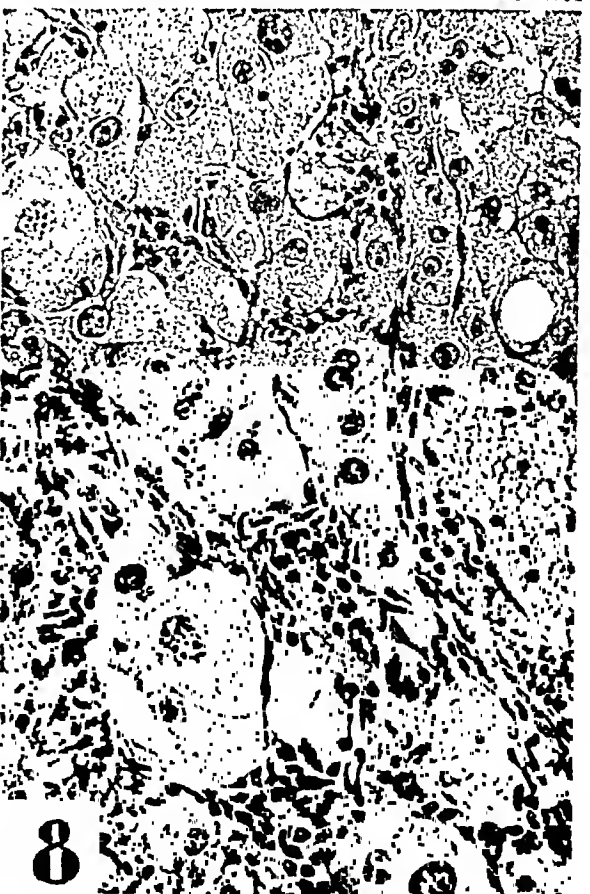
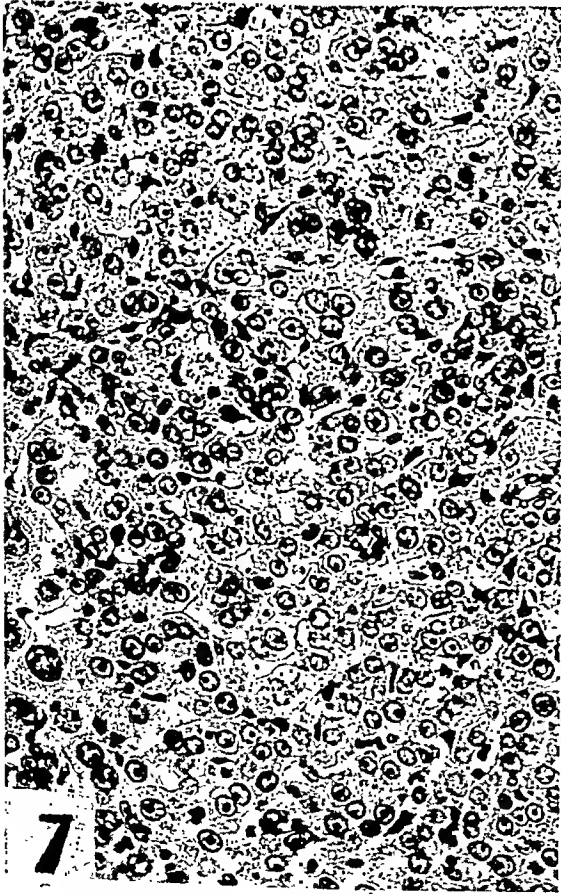
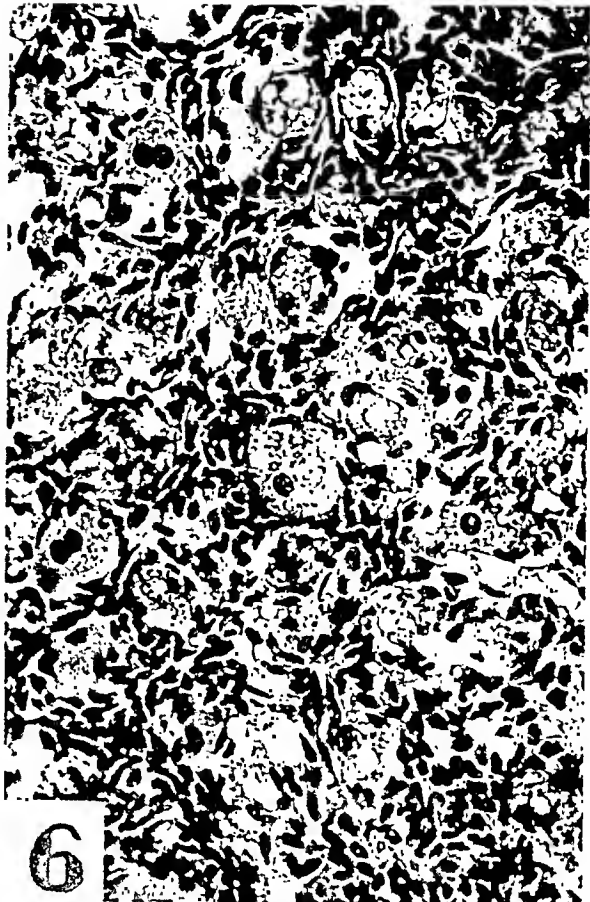
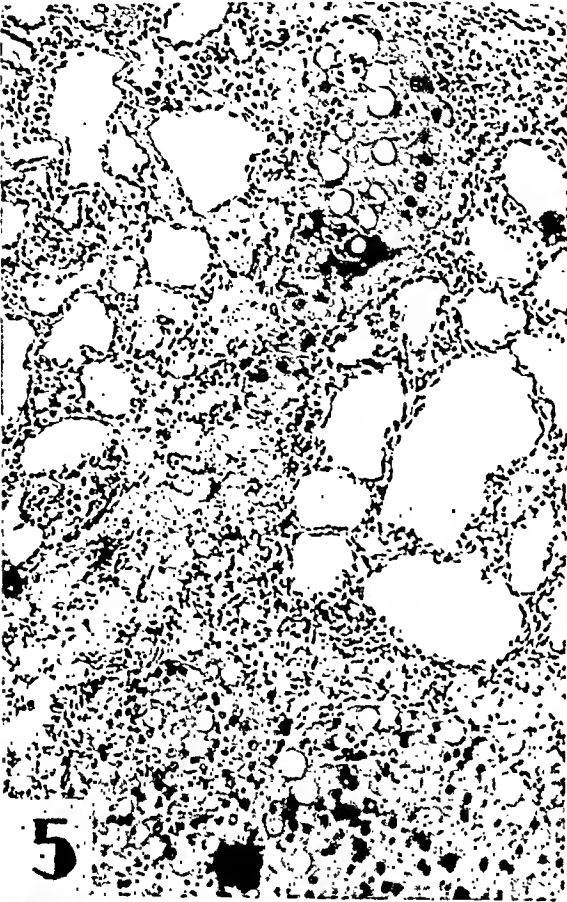


PLATE 203

FIG. 9. Gross dissection showing the appearance of the liver of an animal on Diet 43A for 10 months or longer. The nodules extend down into the connective tissue of the mesentery.

FIG. 10. Neoplasm of the nature of a hepatoma. Of note are the numerous mitotic figures. Hematoxylin and eosin stain.  $\times 275$ .

FIG. 11. Definite cell (cytoplasmic) membranes and peculiar arrangement of cells in a hepatoma. Hematoxylin and eosin stain.  $\times 300$ .

FIG. 12. Cells arranged in a cord-like fashion with wide sinusoid-like spaces between the cords in a hepatoma. Hematoxylin and eosin stain.  $\times 300$ .



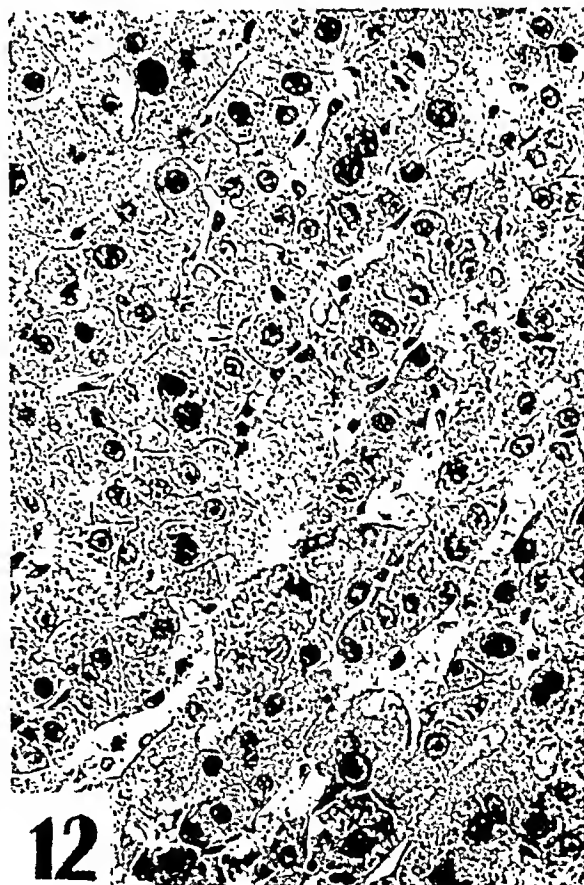
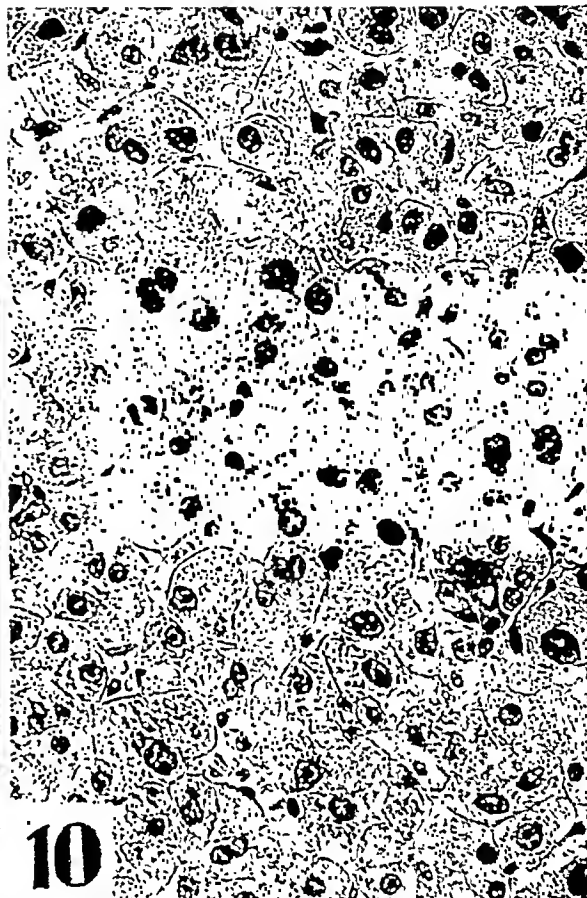




PLATE 204

FIG. 13. Neoplasm of the nature of an adenocarcinoma from liver-like cells within the hepatic lobule. Hematoxylin and eosin stain.  $\times 300$ .

FIG. 14. Neoplasm of the nature of an adenocarcinoma in the interlobular connective tissue of the liver. Hematoxylin and eosin stain.  $\times 145$ .

FIG. 15. An area of more extensive growth of the adenocarcinoma to show the nature and arrangement of the cells and the stroma. Hematoxylin and eosin stain.  $\times 450$ .

FIG. 16. Neoplasm of the nature of a hemangio-endothelioma showing the vascular nature and the type and arrangement of the cells. Growths of this nature were found in the subcutaneous and retroperitoneal fat. Hematoxylin and eosin stain.  $\times 260$ .

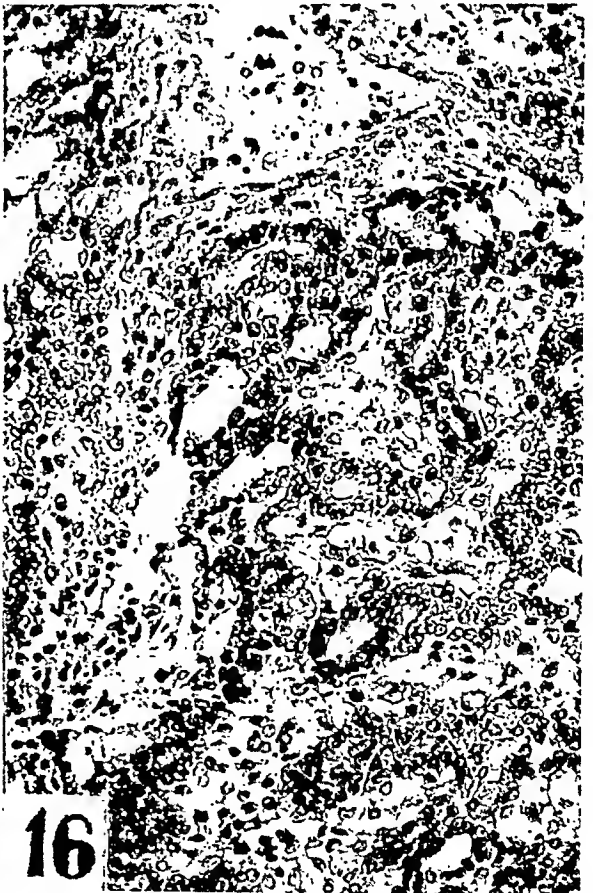
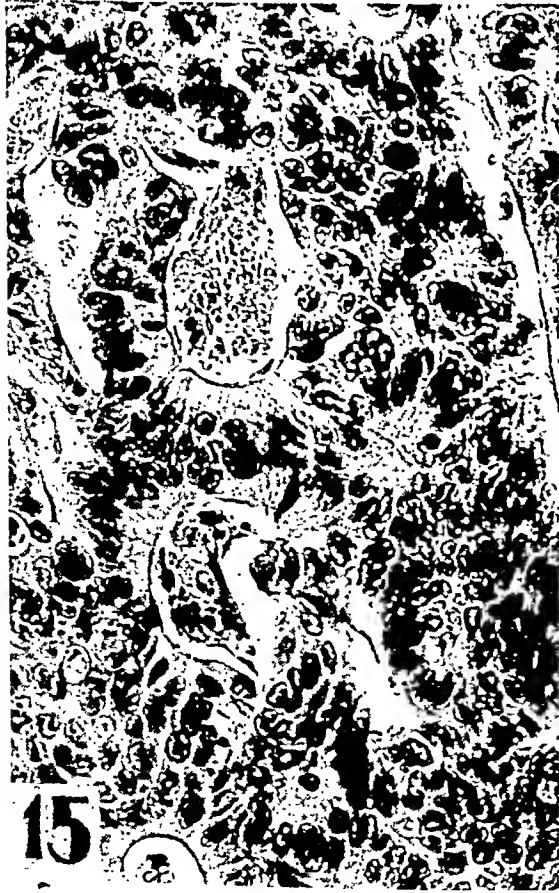
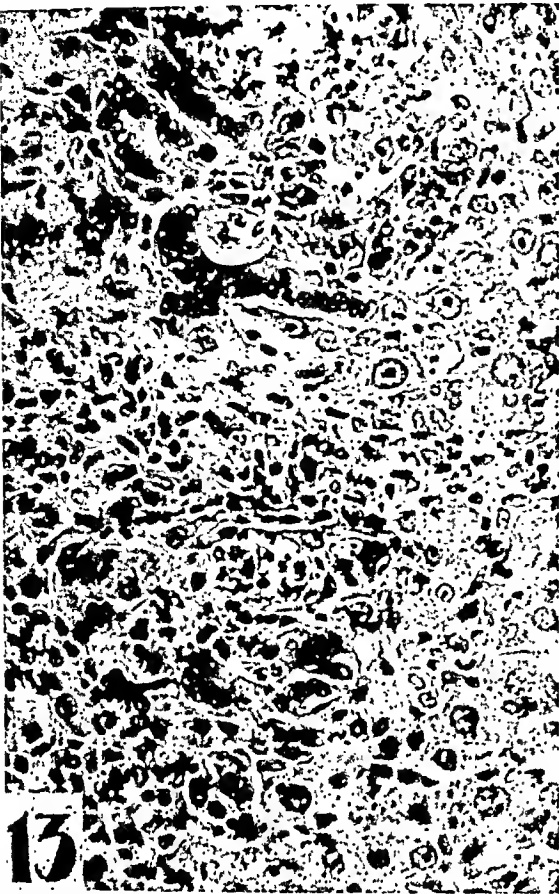


PLATE 205

FIG. 17. Lung of a rat on Diet 43A for 12 months, exhibiting nodular growths of various sizes.

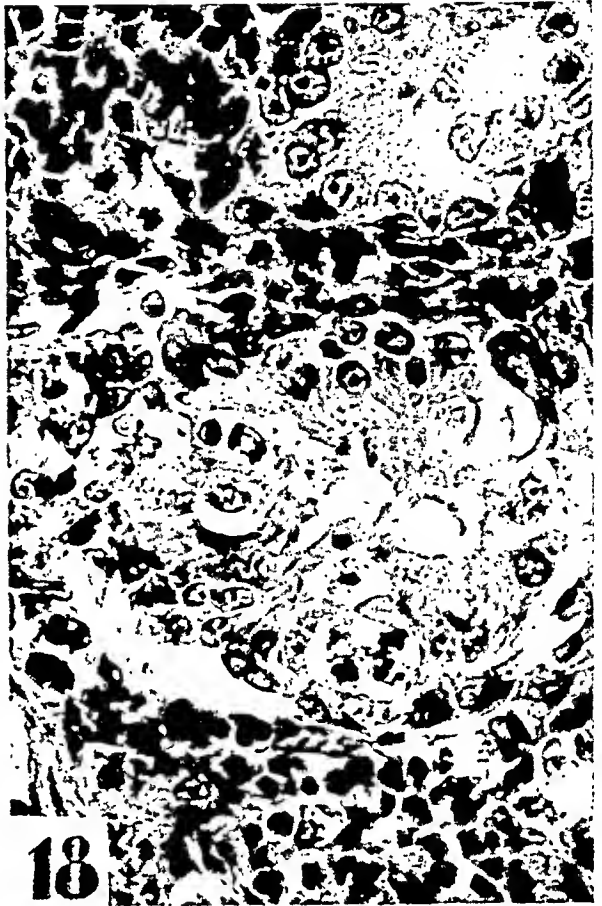
FIG. 18. Neoplasm of the nature of a primary carcinoma in the lung of a rat. Some of the cells have an adenomatous arrangement. Hematoxylin and eosin stain.  $\times 590$ .

FIG. 19. Metaplasia of the epithelium lining a bronchiectatic cavity. The epithelium exhibits a transition from simple columnar to typical stratified squamous epithelium. The neoplasm can be seen underlying the epithelium. Hematoxylin and eosin stain.  $\times 300$ .

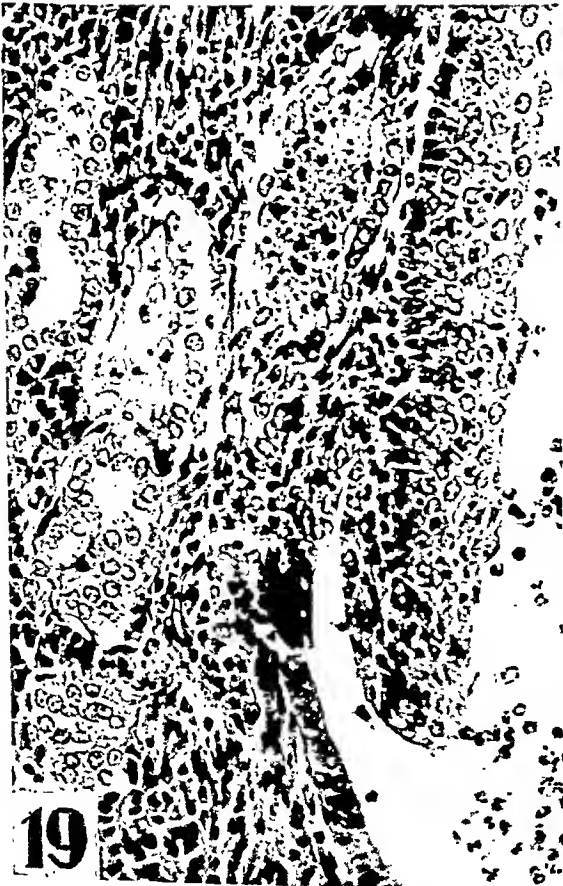
FIG. 20. Papillomatous growth from a lung showing acini-like spaces containing sheets of cells. Hematoxylin and eosin stain.  $\times 300$ .



17



18



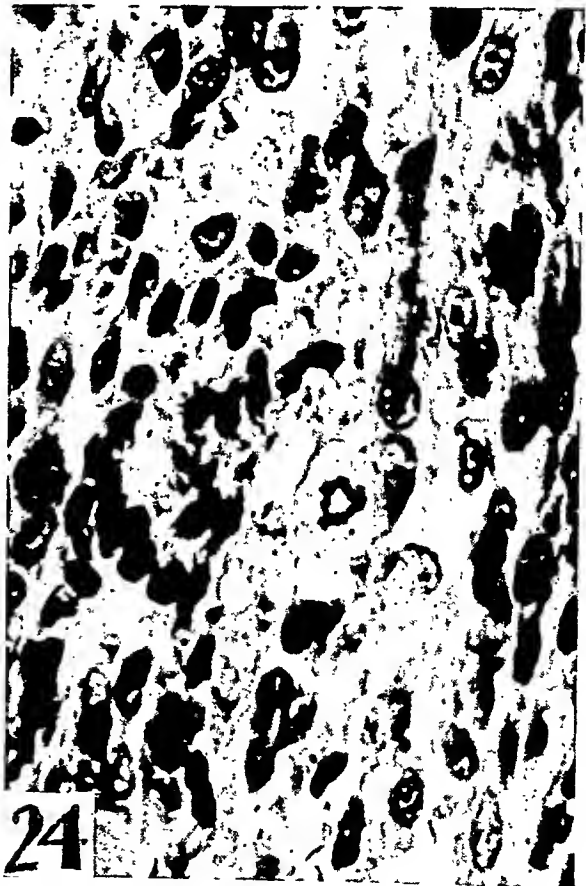
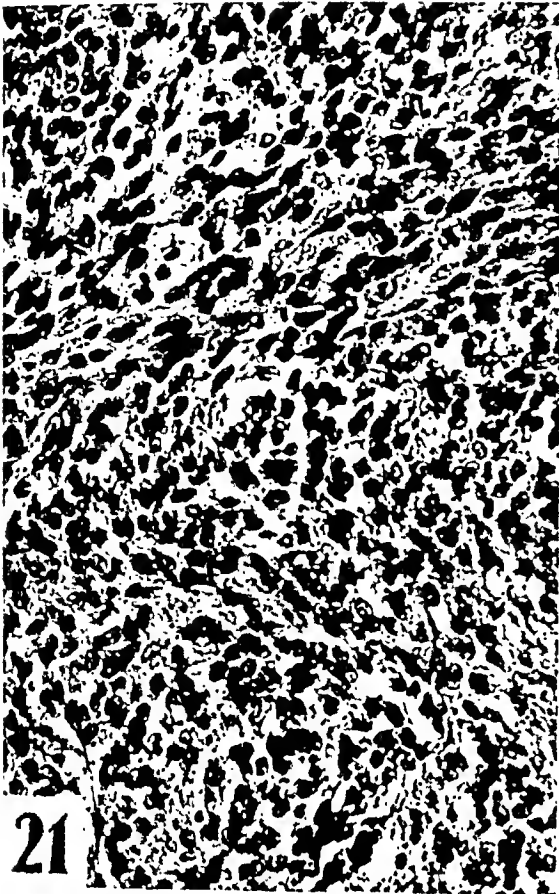
19



20

PLATE 206

- FIG. 21. Neoplasm of the nature of a sarcoma. This growth was retroperitoneal in location. Of note are the cellular nature of this growth and the relatively small amount of connective tissue fibers present. The cells are arranged in planes and are whorled in different directions. Hematoxylin and eosin stain.  $\times 290$ .
- FIG. 22. Neoplasm of the nature of a fibrosarcoma showing a large amount of fibrous tissue. Hematoxylin and eosin stain.  $\times 290$ .
- FIG. 23. Neoplasm of the nature of a sarcoma showing invasion and replacement of muscle. There are numerous mitotic figures. Hematoxylin and eosin stain.  $\times 470$ .
- FIG. 24. Sarcoma enlarged to show the nature of the cells and an atypical mitotic division figure. Hematoxylin and eosin stain.  $\times 820$ .





# THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XXII

NOVEMBER, 1946

NUMBER 6

## FATAL HOOKWORM DISEASE IN INFANCY AND CHILDHOOD ON GUAM \*

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From January 26th through August 9th, 1945, 50 post-mortem examinations were performed on children of 4 years of age and under in the U.S. Naval Military Government Hospital No. 203 on Guam, the Marianas Islands. In 21 instances, the primary cause of death was hookworm disease with its attendant complications. The next most frequent cause of death was tuberculosis, encountered in 5 cases. This rather startling and unique experience seems ample justification for adding another report to the already extensive literature on the subject of hookworm disease. Furthermore, these cases afford a clear view of some of the various mechanisms of the disease which nonfatal cases fail to show.

During the 6½ months which comprise the period of this study, life on Guam for the natives was, indeed, at a low level. Three years of Japanese occupation had so reduced the food supply that nutritional deficiencies, such as the avitaminoses and, more especially, hypoproteinemia, were quite frequent. The battle for possession of the island in the fall of 1944 reduced most of the permanent homes to mere rubble, and by the first of the new year the majority of the native families were living in small one-room huts which were little more than lean-tos. Sanitary facilities were most primitive and often nonexistent. The shallow pits employed as receptacles for excreta were filled to overflowing by the frequent heavy rains. Bathing and washing facilities were difficult to procure both because of excessive crowding in the hastily constructed villages and an inadequate water supply. Clothes, therefore, were soiled and worn for days without being changed. Under such conditions of existence, it is not surprising that nearly 100 per cent of the population was infected with hookworms and had other intestinal parasitism, such as ascariasis and trichuriasis. Of course, as

\* The Bureau of Medicine and Surgery of the Navy does not necessarily endorse the views set forth in this paper.

Received for publication, December 24, 1945.

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sanitary conditions rapidly improved in the latter months of this study, both the incidence and severity of these parasitic infections dropped precipitantly.

Weather conditions on Guam are such as to make hookworm infection a constant threat. The year-round tropical heat and the abundant rainfall meet the conditions necessary for the maturation of hookworm ova into infective filariform larvae. Heavy vegetation affords the requisite shade for this developmental process. Yet 13 of the 21 children with fatal hookworm disease, who comprise the present series, were of an age (under 16 months) which almost precluded acquisition of the infection by the usual method of skin contact with contaminated soil. One of the more puzzling features of the disease was the extreme youth of the victims, but a plausible explanation for this has recently been found. Loughlin and Stoll<sup>1</sup> of this Research Unit have discovered that wearing apparel, and especially bed clothes, when contaminated with infective fecal matter and allowed to remain damp and unwashed for some days, will harbor appreciable numbers of filariform hookworm larvae. They have actually recovered viable larvae from these materials in homes within villages from which our fatal cases originated.

Two factors, in addition to the youth of the patients, were of prime etiologic importance in the production of the fatal hookworm disease. One, already briefly mentioned, was the general state of malnutrition coincident with the Japanese occupation and the subsequent destruction when Americans reoccupied the island. At 3 years of age, the children were still breast fed and received very little supplementary feeding. Green vegetables, meats, and fresh fruits were lacking in the diet. The high incidence of childhood tuberculosis perhaps attests the generalized malnutrition, and, in addition, 2 cases of fatal rickets were discovered in infants during this period. It is, therefore, reasonable to assume that the universal hypoproteinemia was as much the result of antecedent poor nutrition as of the hookworm infection itself.

The other factor was concerned with the species of parasite producing hookworm disease on Guam. Many writers<sup>2,3</sup> have emphasized the greater virulence of *Ancylostoma duodenale* as compared to *Necator americanus*. In view of this, Lieut. N.R. Stoll, H(S), U.S.N.R., undertook an investigation of the species of hookworms found in 11 of the 21 individuals necropsied at the Military Government Hospital. He also examined worms recovered from 7 other infants in the same hospital after anthelmintic treatment. From both sources he obtained a total of 982 hookworms and found that 93 per cent were *Ancylostoma duodenale*.<sup>4</sup> This was the sole species in 13 of the 18 cases. It should be mentioned that not a single *Ancylostoma braziliense* was discovered.

## CLINICAL HISTORIES

The data obtainable from the clinical records of the 21 fatal cases of hookworm disease are woefully inadequate in many respects. This was due to several reasons, not the least important of which were the unsettled conditions on the island at the time during which the study was made. More important, however, was the unexpected suddenness of the fatal termination of the illness in the patients who died in the hospital as well as in their homes. Frequently, there was little or no time in which to make the necessary physical and laboratory examinations. The available clinical data are summarized in Table I.

There was no predominance of the specific infection in female children as compared to male. Although it was realized in a number of instances that hookworm disease was the underlying condition, signs of a terminal infection, such as pneumonia, obscured this fact and led to a faulty evaluation of the primary and secondary causes of the illness. The short period of time during which most of the patients were under observation often precluded accurate differential diagnosis. It must be admitted, too, that the several physicians under whose observation these patients came were not fully aware of the possibility of hookworm disease assuming such a fulminating and fatal course.

It was difficult to determine with accuracy the duration of the terminal illness, and in 8 instances even an approximation could not be made. For the most part, these were the children who died at home. Careful inquiry of the respective families failed to elicit more than the observation that the child had been pale "for some time" but was not considered alarmingly ill. In most instances where the duration of the terminal illness was succinctly specified, it merely indicated when the condition took a decided turn for the worse.

The unusual findings on laboratory examination of cases 4 and 8 need further comment. Verification was made of both the red and white blood cell counts in the former case, and a repeated count of the leukocytes after 8 hours disclosed 98,000 cells with essentially similar differential values. The surprisingly high number of lymphocytes (92 per cent) in case 8 may be related to the nonsuppurative interstitial pneumonia found at necropsy.

*Post-mortem Findings*

Those circumstances which hindered the acquisition of adequate clinical data in the fatal cases of hookworm disease fortunately did not interfere with complete post-mortem examinations. A summary of the findings at necropsy is presented in Table II.

TABLE  
Summary of Clinical Data in

Case no.	Sex	Age (months)	Clinical diagnosis	Duration of terminal illness	Admission temperature	Pallor	Diarrhea	Vomiting	Cough
1	F	11	?	8 hours	100.5°-107°F.	6 mos.	—	—	—
2	M	3	Hookworm disease	5 days	99.5°	+	—	—	+
3	M	7	Pneumococcal meningitis	7 days	99°-103°	+	—	—	—
4	M	6	?	14 hours	104.5°	+	—	—	—
5	F	10	?	8 hours	101.5°	1 wk.	—	—	+
6	F	11	Hookworm disease	1 day	102.4°	—	4 days	+	—
7	F	12	Otitis media	2 wks.	98.6°	+	3 days	—	—
8	M	7	Hookworm disease; pneumonia	4 hours	100.0°	1 mo.	+	+	—
9	M	8	Hookworm disease; pneumonia	1 day	100.0°-107°	2 wks.	—	—	+
10	F	24	Bronchopneumonia	—	101.0°	+	—	—	—
11	M	7	? (Died at home)	—	—	—	—	—	—
12	F	36	Bronchial asthma	18 hours	99.5°-103°	—	—	—	2 wks.
13	F	48	Peritonitis	12 hours	101.5°	—	2 days	+	—
14	M	14	Lobar pneumonia	13 hours	101.4°	+	—	—	2 days
15	F	24	? (Died at home)	—	—	—	—	—	—
16	M	24	? (Died at home)	—	—	—	—	—	—
17	M	36	? (Died at home)	—	—	—	—	—	—
18	F	36	Peritonitis; pneumonia	4 hours	107.0°	+	—	+	+
19	F	16	? (Died at home)	—	—	—	—	—	—
20	F	48	? (Died at home)	—	—	—	—	—	—
21	M	8	Hookworm disease	—	104.0°	1 mo.	—	+	—

Listed under malnutrition are 6 cases which represented rather severe degrees of physical underdevelopment and emaciation. For example, a child of 3 years of age, case 12, appeared hardly older than a 1-year-old child. With but 2 exceptions, the remaining 15 children were borderline cases of poor nutrition.

The diagnosis of anemia was based on several factors. One was the pale appearance of the mucous membranes and of the skin. The latter, because of the natural cutaneous pigmentation of the native Chamorro, had a striking "café au lait" color in the presence of anemia. More important, however, was the pale pink color and almost watery consistency of the blood. Also, samples of bone marrow removed from the sternum, ribs, vertebrae, and femur were pale and hypoplastic.

Accumulations of excessive amounts of fluid in the subcutaneous tissues, abdomen, chest, and pericardium were associated with one or more of the following findings: malnutrition, anemia, and cardiac dilatation. In 3 instances where there were such fluid accumulations in the presence of cardiac hypertrophy and dilatation, there was also evidence of congestive heart failure. This was indicated by the presence of chronic passive congestion in the lungs, liver, and spleen. One of the remarkable features presented by 7 of the fatal cases was the cardiac

I

*Cases of Fatal Hookworm Disease*

Physical examination			Laboratory examinations						
Lungs	Heart	Abdomen	Stool	Blood					
			Hookworm ova	Red blood cells	Hemo-globin	White blood cells	Seg-mented forms (%)	Lymph-ocytes (%)	Eosino-phils (%)
Râles	Enlarged	Distention	+						
—		—		1,920,000	35%	10,850	70	19	9
Rapid respiration		—		2,270,000	7gm.	45,850	74	18	
Dyspnea	Enlarged	Soft	+	980,000	15%	102,900	40	32	12
Consolidation				1,940,000	30%	14,000	64	18	16
Grunting respiration					1.5gm.	13,900	8	92	
Consolidation					2.5gm.	22,640	37	61	
Dyspnea; cyanosis					5.0gm.	12,200	60	30	4
Râles	Tachycardia	Soft	+						
Dullness									
Irregular resp.	Tachycardia	Distention	+						
		Rigid		2,820,000	6.0gm.	10,900	55	34	4

enlargement. This was due to chronic dilatation alone in 4 instances and to both hypertrophy and dilatation in 3. The myocardium was invariably pale and had the yellow mottling associated with anemia. The chambers were greatly enlarged; the papillary muscles were prominent from hypertrophy but were also flattened (Fig. 1).

It has already been mentioned that a terminal febrile illness frequently confused the clinical picture and caused the underlying hookworm infection to be overlooked. In 7 instances, the terminal infection involved the lungs in a nonsuppurative interstitial inflammatory process indistinguishable from a viral pneumonia. In 3 additional cases there was an ordinary bronchopneumonia, with the inflammatory leukocytic exudate dispersed as small foci which were often in peribronchiolar locations. Another patient, case 14, had pneumococcal lobar pneumonia with fibrinopurulent pleurisy. Case 1 had a widespread hemorrhagic pneumonia of undetermined etiology. Finally, there were 2 instances of bronchial involvement. One was an ordinary purulent tracheo-bronchitis (case 13) and the other (case 12) was a bronchiolitis with plugs of mucus, hyalinization of the basement membrane, and peribronchiolar eosinophilic infiltration. It was the latter case which was diagnosed clinically as bronchial asthma, and the anatomic findings

seemed to support this diagnosis. Thus, 14 patients of this series had infections of the respiratory tract complicating hookworm disease.

Apart from inflammatory changes, the lungs were altered in other ways in 3 patients. One, case 15, had multiple fresh hemorrhages, and 2 others, cases 3 and 6, had fibrosis. The presence of various types of débris in the alveoli and in the capillaries of the alveolar walls in these and other cases suggested fragmented or disintegrating hookworm larvae. Accordingly, a careful search was made through many microscopic preparations of the lungs of these patients, but no well formed, identifiable larvae were found. Some of the débris, nevertheless, had the colorless, translucent appearance of larval sheaths. To permit comparison between the material found in the lungs of some of the patients and actual hookworm larvae, a cat was infected with *A. duodenale* by having many filariform larvae placed on the pads of its feet. At the end of 6 days, numerous parasites, as well as an inflammatory cellular reaction, were found in the pulmonary alveoli (Fig. 18). The débris which resulted from disintegrating larvae was closely similar to that seen in the human lungs, yet positive identification could not be made in the latter. The pulmonary fibrosis and the focal hemorrhages may possibly represent injuries which resulted when the larvae penetrated the alveolar septa to enter the acini.

Other serious infections obscured the underlying hookworm disease. In case 3, a 7-months-old male infant who had evidence of severe hookworm disease, there developed an acute otitis media which progressed to mastoiditis and terminated in pneumococcal meningitis. A female infant, 11 months old, case 6, developed a serious thrush infection of the pharynx. The outstanding inflammatory lesions, however, affecting 18 of the 21 cases, were the result of the hookworm infection itself and involved the jejunum and upper part of the ileum. In 6 of these cases, an extensive fibrinopurulent peritonitis had also occurred.

The involvement of the jejunum invariably began at the fossa of Treitz and extended down for a variable distance in the ileum, rarely affecting more than the first 50 cm. The duodenum was involved only once or twice to the extent of harboring a few hookworms. Macroscopic inspection of these parts of the small intestine disclosed all gradations of involvement ranging from little more than occasional petechiae to gangrene and extensive hemorrhage of the jejunum, with fibrinopurulent peritonitis, in the most severely involved cases (Fig. 2). The intestinal wall was greatly thickened, but in no instance was it actually perforated. Examination of the mucosal surface of the jejunum frequently disclosed many adult hookworms attached to the villi (Fig. 3). Petechial hemorrhages in the mucosa were found at sites

TABLE II  
Summary of Necropsy Findings in Fatal Cases of Hookworm Disease

Case no.	Malnutrition	Anemia	Lymphadenitis	Eosinophilia in bone marrow	Anasarca	Hydrothorax	Hydropericardium	Ascites	Chronic passive congestion of viscera	Heart	Lungs	Intestines	Miscellaneous findings	Hookworms	Ascaris	<i>Trichuris trichiura</i>
1	+	+			+	+	+		+	Hypertrophy and dilatation	Hemorrhagic pneumonia Aspiration of gastric contents Fibrosis	Acute enteritis Acute jejuno-ileitis; blood Focal jejunitis; blood	Otitis media; meningitis Focal hepatitis and fibrosis	20+	-	-
2		+	+											99	-	-
3		+	+		+	+	+		+	Dilatation	Interstitial pneumonia Aspiration of milk	Petechiae and ulcers of jejunum Ulcerative jejuno-ileitis		107	-	-
4		+	+				+				Interstitial fibrosis	Ulcerative jejuno-ileitis	Thrush pharyngitis	60+	-	-
5	+		+								Interstitial pneumonia	Ulcerative jejuno-ileitis		200+	-	-
6	+		+								Interstitial fibrosis	Ulcerative jejuno-ileitis		24	1	-
7		+	+			+		+	+	Hypertrophy and dilatation	Interstitial pneumonia	Acute jejuno-ileitis		150+	1	-
8		+	+		+	+					Interstitial pneumonia	Acute jejuno-ileitis		200+	-	-
9		+	+		+						Edema	Acute jejunitis	Acute pericholangitis	250	-	-
10		+	+								Focal pneumonia	Petechiae in ileum		50	-	+
11		+	+			+					Focal pneumonia	Acute jejuno-ileitis		7	1	-
12	+	+	+								Acute bronchiolitis		Pulmonary emphysema and atelectasis	100+	3	+
13		+	+								Tracheobronchitis	Gangrene of jejunum	Purulent peritonitis	75	31	+
14	+	+	+			+				Dilatation	Diffuse pneumonia; pleurisy Multiple hemorrhages	Ulcerative jejunitis		50+	-	-
15		+	+								Focal interstitial pneumonia	Suppurative jejunitis	Purulent peritonitis	70+	27	-
16			+									Necrotizing jejunitis	Purulent peritonitis	20+	-	-
17			+									Necrotizing jejunitis	Purulent peritonitis	100+	-	-
18		+	+									Necrotizing jejunitis	Organizing peritonitis	43	1	48
19		+	+		+	+						Necrotizing jejunitis	Purulent peritonitis	200+	2	+
20								+		Hypertrophy and dilatation	Focal pneumonia	Necrotizing jejunitis	Purulent peritonitis	50+	-	-
21	+	+	+							Dilatation	Interstitial pneumonia	Necrotizing jejunitis	Dehydration	10+	-	-

where parasites were no longer attached. Many worms were also found free in the fecal contents. In several instances, large quantities of partially changed blood were mixed with the feces. As a rule, the mesenteric lymph nodes of the most heavily infected patients were greatly enlarged, pale, and succulent.

On microscopic examination, the jejunum of every patient was infiltrated with polymorphonuclear neutrophilic leukocytes and with eosinophils in various proportions. Sometimes this cellular reaction was limited to the mucosa, at other times it extended to involve the submucosa, and at still other times it infiltrated the muscularis as far as the serosa (Fig. 4). Serial microscopic preparations of the jejunum disclosed pieces of mucosa clamped in the mouth parts of hookworms (Fig. 5). In some instances, the head of a parasite had evidently burrowed through the mucosa and penetrated the submucosa (Fig. 6). Superficial mucosal ulcerations, oftentimes exceedingly numerous, marked former sites of attachment of hookworms (Fig. 7). Almost invariably there was still evidence of bleeding in these ulcers, and the subjacent wall was heavily infiltrated with leukocytes. In a few instances, all layers of the intestinal wall contained enormous numbers of eosinophils (Fig. 8). The great thickness and leathery consistency of the jejunum were due in part to the cellular infiltration and in part to a peculiar edema which was most prominent in the submucosa (Fig. 9). Lymphocytes and neutrophilic leukocytes surrounded the submucosal lymphatics as evidence of an extensive lymphangiitis. All too frequently, sites adjacent to areas where the worms were attached were deeply ulcerated and secondarily infected (Fig. 10). This mechanism undoubtedly accounted for the frequent presence of abscesses in the submucosa (Fig. 11). The 6 cases of peritonitis had the most extensive lesions in the small intestine. Not only was the mucosa extensively ulcerated, but there were necrosis, hemorrhage, and leukocytic reaction in all coats (Figs. 12 and 14). Extensive thrombo-angiitis of the vessels in the submucosa accounted for part of the microscopic picture of gangrene. Large vessels, arterioles as well as venules, had necrotic walls even when they were not thrombosed (Fig. 13). Less involved portions of the gut often had zones of hyaline necrosis (Zenker's) of the muscle layers (Fig. 15).

Microscopic study of the attached hookworms themselves disclosed an interesting fact. Their intestinal canals contained bits of ingested mucosal tissue as well as leukocytes and erythrocytes. None of these cellular elements appeared to be altered by their passage from the buccal end of the parasite's intestinal canal to its anal orifice. In Figure 17

are portrayed such unaltered leukocytes and red blood cells in the terminal end of the intestine of a hookworm.

The greatly enlarged mesenteric lymph nodes had widely dilated marginal lymphatics and contained many macrophages filled with phagocytosed white and red blood cells and other débris. A conspicuous feature of all nodes was the presence of numerous eosinophils. Cells of the latter variety were also present in large numbers in the splenic pulp.

In 8 cases there were changes in the bone marrow which could have accounted, at least in part, for the severity of the anemia. These changes consisted of an eosinophilia that replaced the cells of the erythropoietic series (Fig. 16). Eosinophilic myeloblasts and myelocytes were present in large numbers, in addition to mature eosinophilic leukocytes. Conversely, there were few megakaryocytes and erythroblasts. As evidence of this deficiency in hematopoiesis on the part of the bone marrow was the presence of megakaryocytes in both the pulmonary capillaries and the spleen. In 2 of the cases, indeed, there was also evidence of erythrocyte formation in the liver.

### DISCUSSION

The combination of extreme youth of the patients, their malnutrition and neglect, opportunities for reinfection, and the virulent species of hookworm involved, namely *Ancylostoma duodenale*, accounted for the malignant form which the intestinal parasitism assumed in this series of cases. It seems fair to assume that the simultaneous infection with *Ascaris lumbricoides* and with whipworms (*Trichuris trichiura*) contributed but little to the fulminating course, especially since only 2 children had moderately heavy infections with *Ascaris* and only 5 of the children were infected with whipworms. The unusual penetrating proclivities of the hookworms in this series of cases, as shown in Figure 6, are suggestive of the behavior of *Ancylostoma braziliense*.<sup>5</sup> That this species of helminth had no part in the production of the intestinal disease seems apparent from the study undertaken by Lieut. Stoll to which reference has already been made.<sup>4</sup>

Most writers agree with Rhoads, Castle, Payne, and Lawson<sup>6</sup> that the anemia of hookworm disease is the result of blood loss. This occurs from the numerous bleeding ulcerations in the intestinal mucosa, as well as through the intestinal tracts of the attached hookworms. The presence of large quantities of blood in the intestinal lumina of our patients amply confirms this as a prime mechanism of the anemia. But the additional finding of eosinophilia in the bone marrow, with the



consequent interference of normal erythropoiesis, is evidence of the fact that the anemia of the more severe cases is not due alone to blood loss.

### SUMMARY

Twenty-one fatal cases of hookworm disease in early childhood on Guam were investigated.

Malnutrition, neglect, and poor sanitation were considered important factors in this condition.

It was suggested that bed clothes contaminated with filariform larvae of *Ancylostoma duodenale* were the means by which at least some of the children became infected.

Serious terminal infections, chiefly of the respiratory tract, often obscured the underlying hookworm disease.

The outstanding lesion in the condition was acute jejunitis, which progressed in some cases to produce widespread ulceration, hemorrhage, necrosis, and even gangrene of this part of the intestinal tract.

In 6 cases there occurred an extensive purulent peritonitis.

A prominent feature of the intestinal lesion was the infiltration of eosinophilic leukocytes.

Associated with severe anemia, 7 cases had cardiac hypertrophy and/or dilatation.

There was evidence indicating that the anemia was due in part to eosinophilic accumulations in the bone marrow which interfered with normal erythropoiesis.

Acknowledgment is made of the valuable assistance of Robert H. Jackson, Pharmacist's Mate, 2nd Class, U.S.N.R., in histologic technic.

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[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

All photomicrographs were made from preparations stained with hematoxylin and eosin, unless otherwise indicated.

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### PLATE 207

- FIG. 1. Case 19. Female infant, 16 months of age. Cardiac hypertrophy and dilatation. Weight of heart, 95 gm.
- FIG. 2. Case 13. Gangrene of the jejunum with purulent peritonitis.
- FIG. 3. Case 9. Numerous hookworms attached to the jejunal mucosa.
- FIG. 4. Case 2. Infiltration of neutrophilic and eosinophilic leukocytes in all coats of the intestinal wall.  $\times 70$ .
- FIG. 5. Case 5. Hookworm with a piece of intestinal mucosa in its mouth.  $\times 70$ .
- FIG. 6. Case 12. Head of a hookworm impinging on edematous submucosa of the jejunum.  $\times 70$ .

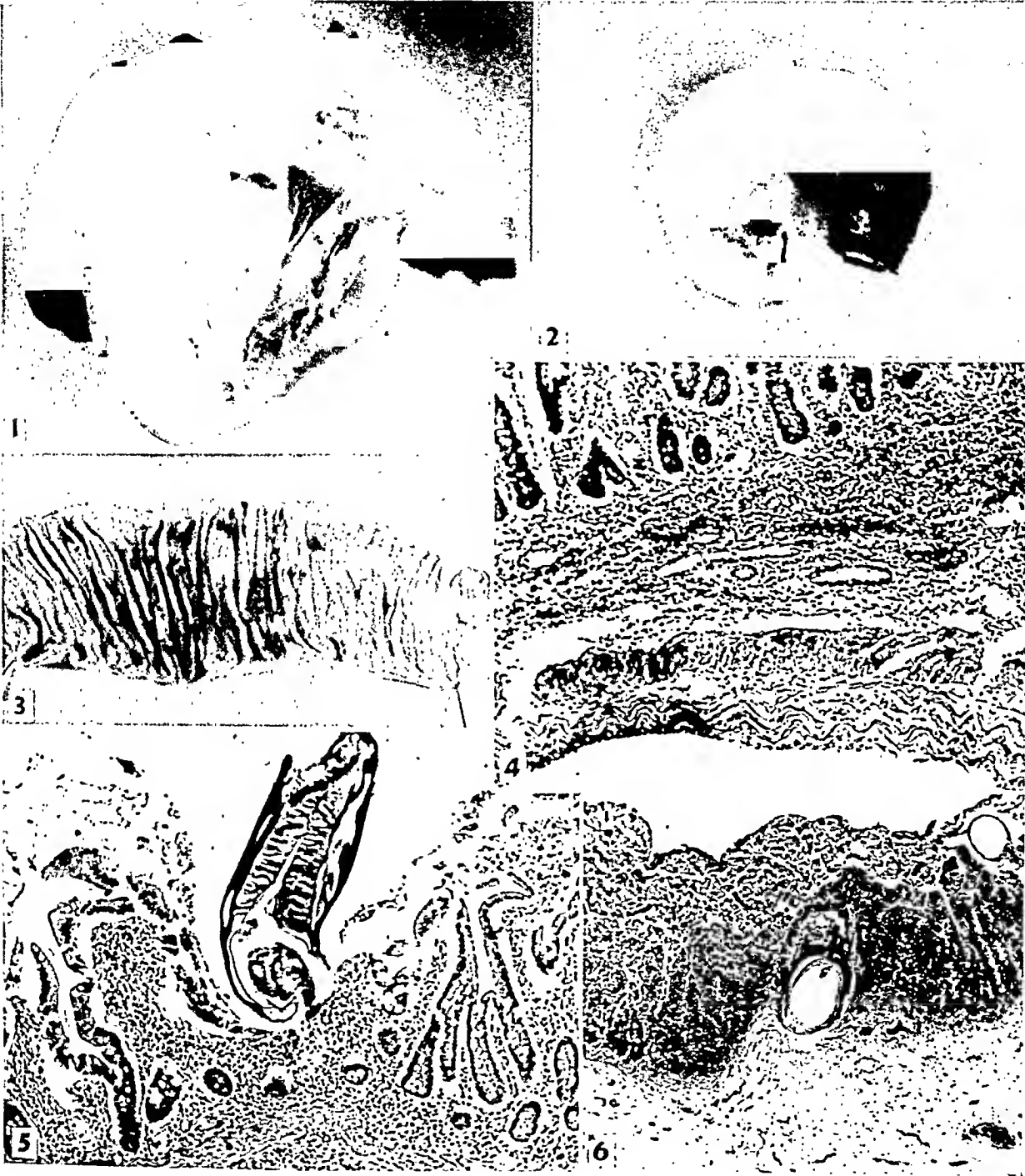


PLATE 208

FIG. 7. Case 19. Cross-section of a hookworm adjacent to superficial mucosal ulceration. Of note is the extensive leukocytic infiltration in the submucosa.  $\times 80$ .

FIG. 8. Case 4. Cells in both the mucosa and submucosa of the jejunum are almost entirely eosinophils.  $\times 80$ .

FIG. 9. Case 19. Massive edema and lymphangiitis in the submucosa of the jejunum.  $\times 80$ .

FIG. 10. Case 14. Ulcerative lesion adjacent to the site of a hookworm attachment.  $\times 80$ .

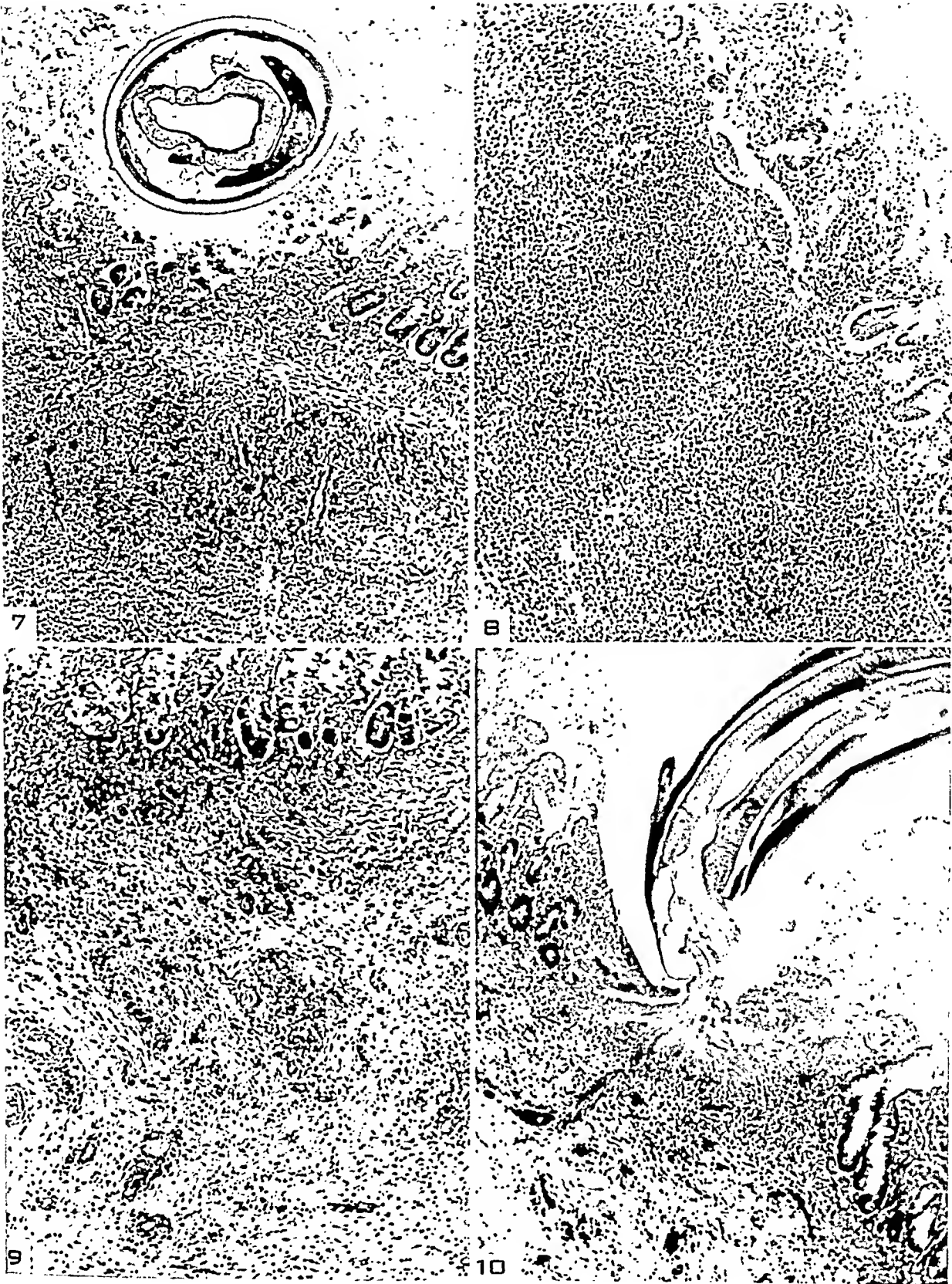


PLATE 209

FIG. 11. Case 6. Abscess in the submucosa of the jejunum. The dark area on the left represents hemorrhage.  $\times 80$ .

FIG. 12. Case 15. Mucosal ulceration, hemorrhage, leukocytic infiltration, and thrombo-angiitis in the jejunum.  $\times 80$ .

FIG. 13. Case 20. Necrosis of the walls of an arteriole and a venule in the submucosa of the jejunum.  $\times 285$ .

FIG. 14. Case 13. Necrotizing and suppurative jejunitis.  $\times 80$ .

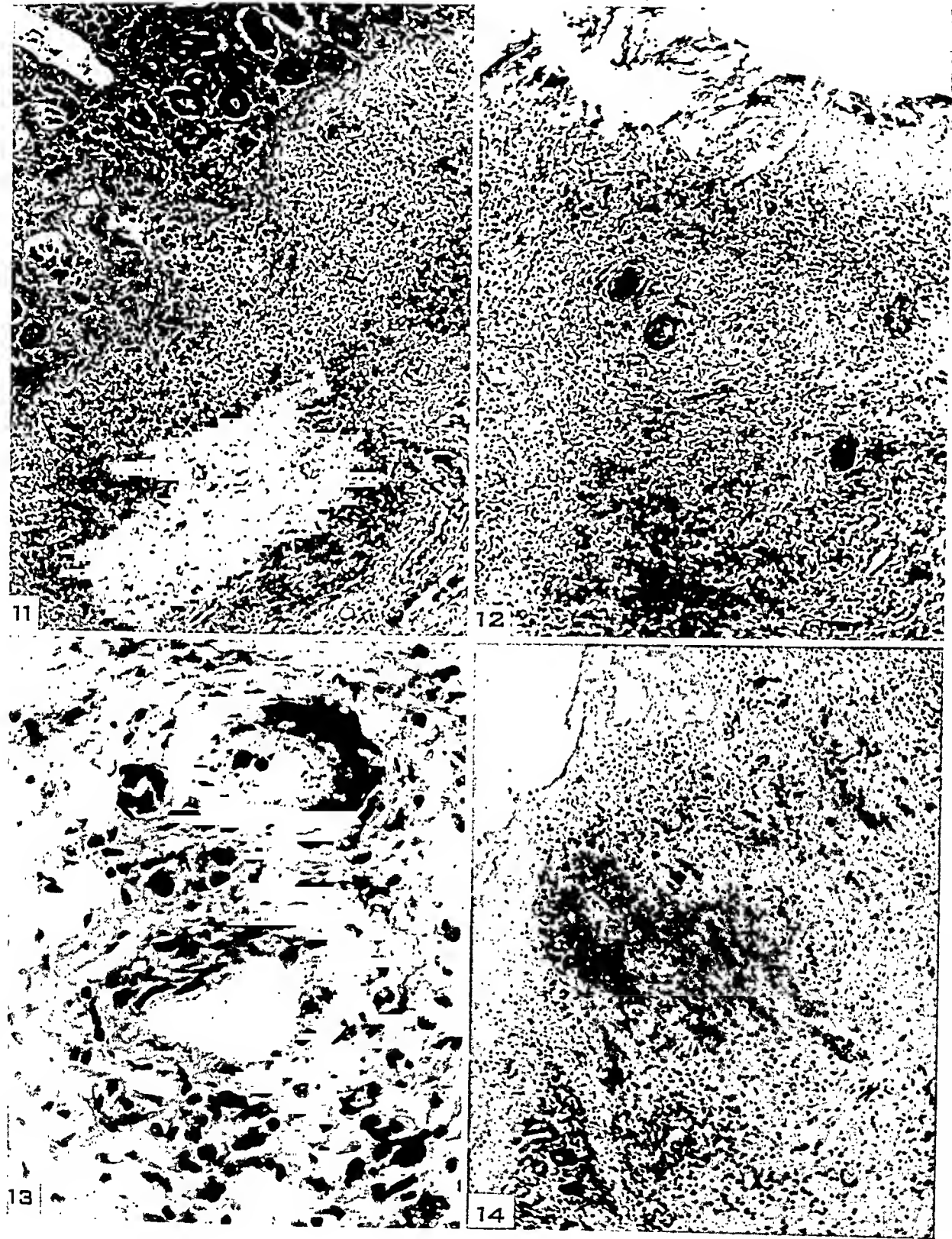
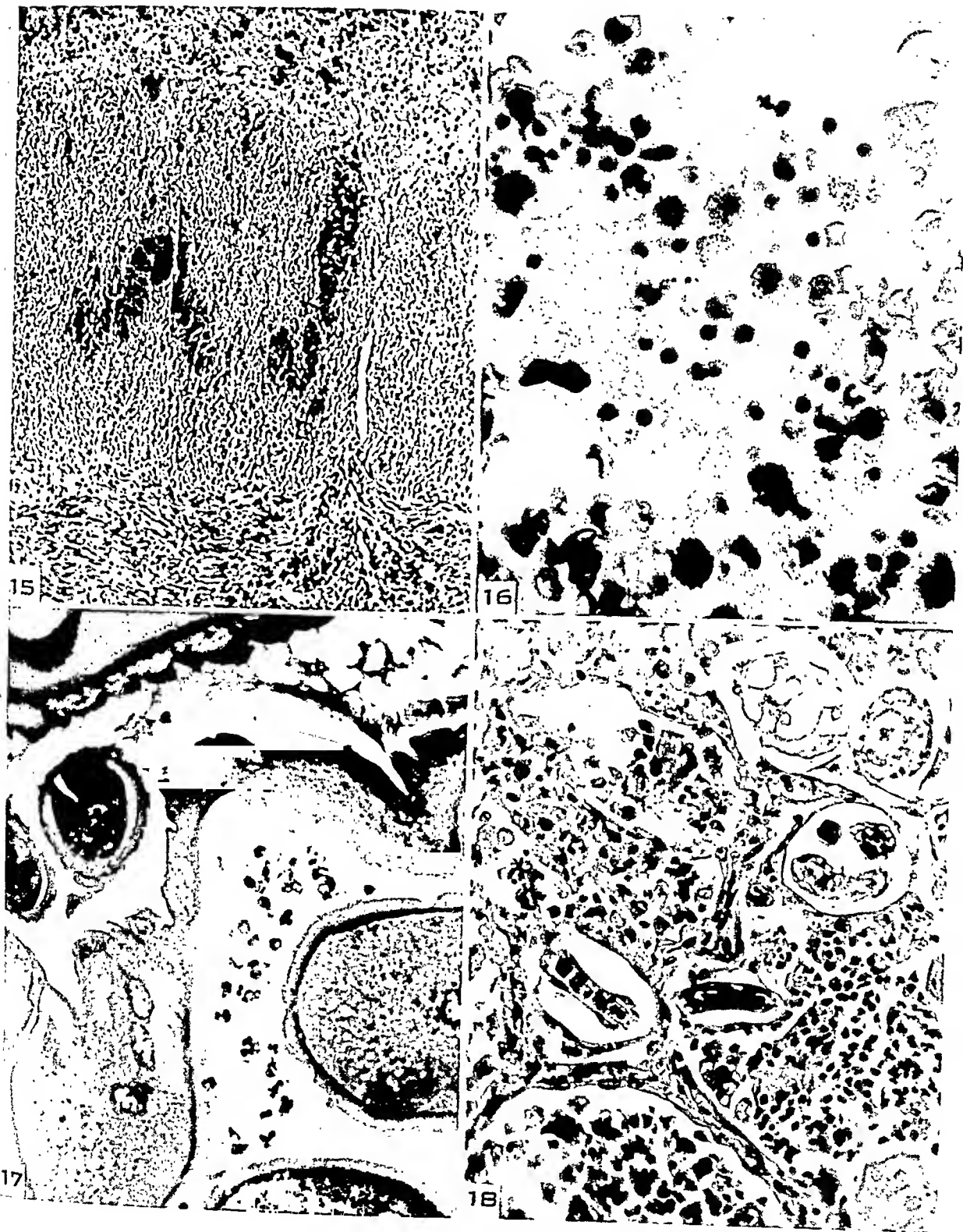




PLATE 210

- FIG. 15. Case 15. Zenker's necrosis in the muscle layer of the jejunum.  $\times 80$ .
- FIG. 16. Case 13. Eosinophils (with large granules) in the bone marrow. Giemsa's stain.  $\times 735$ .
- FIG. 17. Case 19. Intact erythrocytes and leukocytes in the lower intestinal tract of a hookworm.  $\times 285$ .
- FIG. 18. Lung of a cat infected with filariform larvae of *Ancylostoma duodenale* 6 days previously. Of note are the larvae, inflammatory cellular exudate, and débris in the pulmonary alveoli.  $\times 285$ .





# EXPERIMENTS ON THE SPREAD OF NEOPLASTIC CELLS THROUGH THE RESPIRATORY PASSAGES\*

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Little is known about the spread of tumors by way of the respiratory passages. The presence of mucus and the ciliary motion of the lining epithelial cells are believed to oppose the spread of neoplastic cells. Willis<sup>1</sup> stated that no experimental work had been reported on the inoculability of the lungs to tumors by way of air passages. He cited numerous alleged instances of implantation metastasis by way of air passages, but concluded that most of these are susceptible to other explanation. According to Willis,<sup>1</sup> Moxon (1869) was the first to report on inhalation metastases from an esophageal cancer, and Godlee from carcinoma of the tongue. However, the occurrence of intrapulmonary implants in a child from laryngeal carcinoma and from an adenocanthoma in a young man can scarcely be doubted. There are numerous reports in the literature on contralateral metastases of pulmonary growth, all of which have been critically reviewed by Willis.

It was, in part, an accidental observation; in part, curiosity, that led us to undertake to verify the possibility of the spread of tumors by way of the respiratory passages. A study of respiratory viruses in mice made with Dr. P. deGara and Miss Dorothy Kley necessitated intranasal instillation of material containing cells from lung tumors. Unexpectedly this resulted in the development of lung tumors. Recently we made post-mortem studies of a patient who had a slowly progressing malignant adenoma of the trachea, and noted tumor nodules surrounding and stenosing several bronchioles in addition to the usual perivascular lymphatic metastases. A specimen taken for biopsy 4 years before death showed squamous metaplasia of the normal ciliary epithelium, which may have been sufficient to break down the barrier to penetration of foreign particles to the alveoli. Tumor cells which reach the alveoli readily proliferate there, as attested by the occasional findings of massive intra-alveolar growth in metastatic tumors of the lung.

In the experiments to be described a few droplets of a suspension of neoplastic cells were instilled into the nostrils of young healthy mice under light ether anesthesia. In several instances the instillation was repeated after 5 or 24 hours, as indicated in Table I. All mice used

\* These investigations have been supported by The Jane Coffin Childs Memorial Fund for Medical Research, The International Cancer Research Foundation, and The Anna Fuller Fund.

Received for publication, December 17, 1945.

were highly susceptible to subcutaneous implantation of the tumor cells.

The results summarized in Table I indicate that the respiratory surfaces of a healthy animal did not bar dissemination of tumor cells, although in many mice all tumor cells introduced were destroyed. Intercurrent infection was slight, if any.

The course and behavior of the neoplastic cells after intranasal introduction varied greatly:

(a) The carcinoma cells (strain 387) produced progressively growing tumors in both lungs, bringing about death by displacement of lung

TABLE I  
*Results of Intranasal Instillations of Neoplastic Cells*

Experiment no.	Neoplasm		No. of injections	No. of mice		Length of life after inoculation	
	Type	Number		Injected	Positive	Positive	Negative
						(days)	(days)
1	Carcinoma of lung	387	1	6	2	K*15, D29	K15-60
			1	6	2	K15, D21	K15-56
2	Carcinoma of lung	387	1	5	1	D*19	D7, K21-35
			1	3	2	D14-24	K31
3	Carcinoma of lung	387	2	5	3	D19-36	K51, K51
			2	6	2	D18-21	D14-32
4	Carcinoma of lung	387	2	5	5	D14-20	K20
5	Carcinoma of lung	387	1	7	1	D20	K104
			2	7	6	D19-57, K23	K23
6	Lymphoid leukemia	559	2	9	9	K17, D18-22	
7	Lymphoid leukemia	1329	1	3	1	D33	K86
8	Lymphoid leukemia	975	2	5	5	K24-29	
9	Myeloid leukemia	1394	2	5	4	D33-44, K42	K42
10	Myeloid leukemia	1446	1	5	1	K76	K76
11	Myeloid leukemia	1446	2	6	6	D34-37, K38	

\* K = killed, D = died.

parenchyma (Figs. 1 and 3). At the time of death, gross examination disclosed metastases in regional lymph nodes only.

(b) The other extreme of invasiveness is represented by malignant lymphocytes of strain 559 which produced systemic disease without leaving any mark at the point of entry into the circulation.

(c) With respect to invasiveness all other malignant cells studied were between the above extremes. They produced massive peribronchial infiltrations with small or minute tumor-like nodules in the lungs (Figs. 4 to 7), particularly in hilar and apical regions, and moderate or extensive involvement of the draining nodes. In many instances the pulmonary infiltrations extended beyond the pleura, as shown in Figure 7, causing massive pleural effusion and occasionally massive pericardial infiltrations as shown in Figure 8. Death was usually caused by generalized leukemia with characteristics peculiar to the strain (*e.g.*, Fig. 9).

Many neoplastic cells doubtless perish after intranasal introduction

and occasionally all cells perish (Table I). Ability to survive under the circumstances as well as the degree of invasiveness are inherent characteristics of the cells.<sup>2</sup>

All but one of the strains used in these experiments produced a typical monomorphous invasive growth in the lungs and elsewhere of cells of the type named. Strain 1446 was an exception. This strain produced, in places, a fairly uniform tumor-like proliferation of myeloid cells (Fig. 10). Frequently, however, retrogressive changes occurred with fibrosis and accumulations of lymphoid cells, and in draining hilar lymph nodes a granulomatous reaction with multinuclear giant cells developed. This phenomenon needs further study. When death occurs with generalized leukemia of this strain there is usually massive infiltration in the liver (Fig. 9) and exsanguinating hemorrhage, probably caused by prothrombin deficiency.

#### COMMENT

That neoplastic cells appear in the sputum is now well established<sup>3</sup> although little use is being made of the diagnostic potentialities of such observations. Very recently, Papanicolaou has been highly successful in demonstrating malignant cells in the sputum of patients.<sup>4</sup> The experiments here described indicate that neoplastic cells in the sputum or in alveoli may lead to bronchiogenic spread.

The sequence of events is determined by the number and character of the malignant cells as well as by the state of the respiratory surfaces. Lymphoid cells of strain 559 penetrated the respiratory surfaces, producing systemic leukemia with no gross pulmonary infiltrations, and killed mice but a few days more slowly than when introduced directly into the circulation. It is now well known that the manifestations of leukemia depend in part on the invasive character of leukemic cells, in part on their portal of entry (site of origin).<sup>2</sup> This is well illustrated by the experiments here described.

Chloroleukemia, strain 1394, has not produced grossly detectable pulmonary infiltrations following intravenous or subcutaneous introduction into hundreds of mice, but it produced massive tumor-like infiltrations in the lungs following intranasal injection. From the lungs the malignant promyelocytes spread to the hilum and distant organs, killing the mice with either generalized myeloid leukemia or thoracic myelomatosis predominant.

None of the leukemic strains used in this study produced peribronchial infiltrations in the lungs following intravenous introduction. The characteristic feature of the pulmonary changes in the animals that received intranasal instillations of neoplastic cells, on the contrary,

consisted of massive, often tumor-like, peribronchial infiltrations. The figures shown amply illustrate the outstanding feature of these changes; namely, the preservation of the bronchial epithelium in most places and its lifting up from the underlying structures by the neoplasm. No conspicuous ulceration of the mucosa of the respiratory tract was seen in the sections examined, and the site of penetration of the malignant cells was not evident.

### SUMMARY

Epithelial surfaces of the respiratory tract are not safe barriers against the dissemination of neoplastic cells. Carcinoma cells instilled into the nostrils of lightly anesthetized mice reached the lungs and produced large tumors. Leukemic cells of a lymphoid strain similarly introduced produced fatal leukemia with no gross pulmonary changes. Leukemic cells of four other strains (two myeloid, and two lymphoid) produced tumor-like pulmonary infiltrations, with spread to regional lymph nodes terminating in generalized leukemia.

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### DESCRIPTION OF PLATES

All sections are from mice that received intranasal instillations of neoplastic cells. The sections were stained with hematoxylin and eosin. The magnifications given are approximate.

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### PLATE 211

- FIG. 1. Lungs of two mice that died following intranasal instillation of cells of a tumor of the lung, strain 387. Slight magnification.
- FIG. 2. Lungs of a mouse that died following intranasal instillation with leukemic lymphocytes of strain Akl 975, showing massive mediastinal lymphomatous infiltrations. Slight magnification. Dorsal view of both lungs.
- FIG. 3. Infiltrating neoplasm in the lung of a mouse which died 19 days following intranasal instillation of lung carcinoma cells from strain 387.  $\times 100$ .
- FIGS. 4 and 6. These photomicrographs are from a mouse that died 42 days following intranasal instillation of malignant myelocytes of strain 1394.  $\times 100$ . In this and other pictures the bronchial mucosa is lifted away from the underlying submucosa by massive neoplastic infiltrations.
- FIG. 5. Lung of a mouse which died 35 days following intranasal instillation of cells from myeloid leukemia, strain 1446.  $\times 100$ .

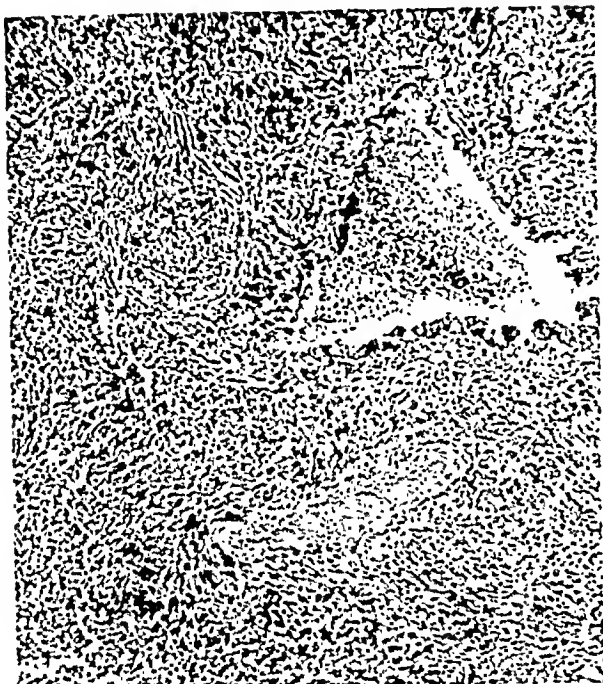
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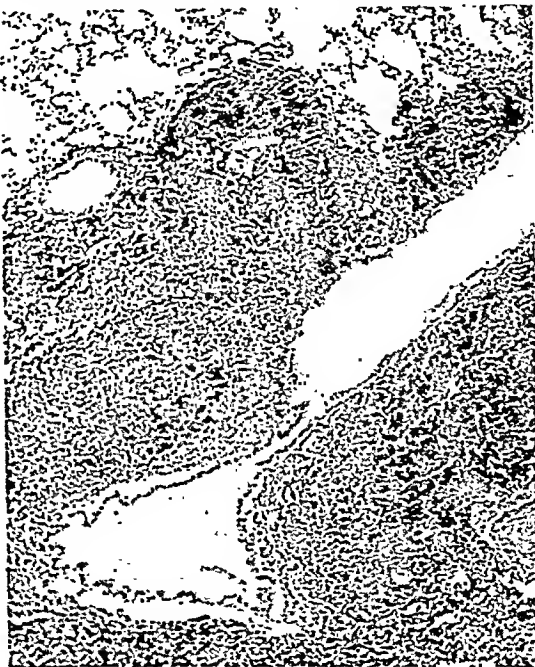
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6



Furth

Neoplastic Cells in the Respiratory Passages



PLATE 212

Figures 7 to 10 are of organs of mice which died following intranasal instillation with myeloid leukemia, strain 1446.

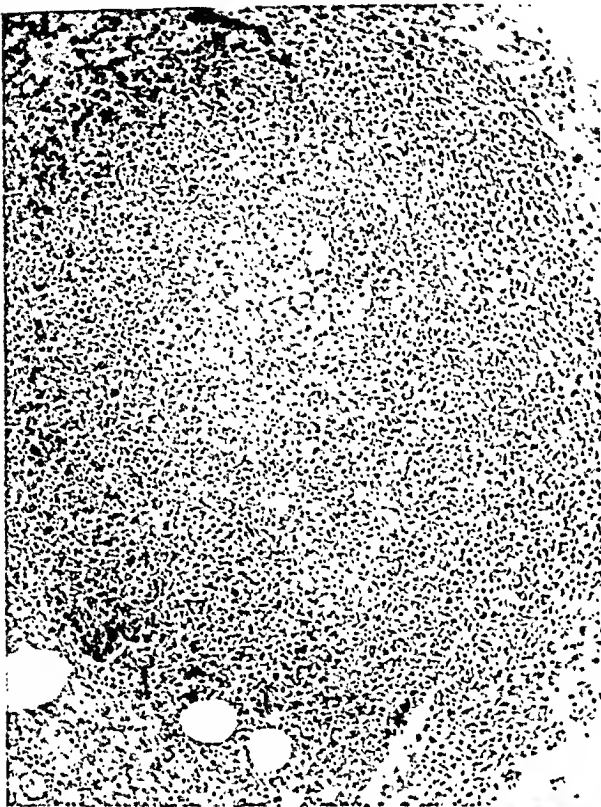
FIG. 7. A nodule of myelomatous infiltration in the lung of a mouse that died 37 days following intranasal instillation. There is central necrosis and extension of leukemic infiltration into the pleural space.  $\times 100$ .

FIG. 8. Massive pericardial infiltration in the same mouse as shown in Figure 7.  $\times 100$ .

FIG. 9. Massive leukemic infiltration in the liver of a mouse that died 34 days following intranasal instillation.  $\times 180$ .

FIG. 10. Higher power view ( $\times 900$ ) of myelomatous mediastinal infiltration in the lung of a mouse that was killed 38 days following intranasal instillation. Of note are the mitotic figure and the pale-staining cells with doughnut-shaped or indented nuclei—characteristic myelocytes of the mouse.

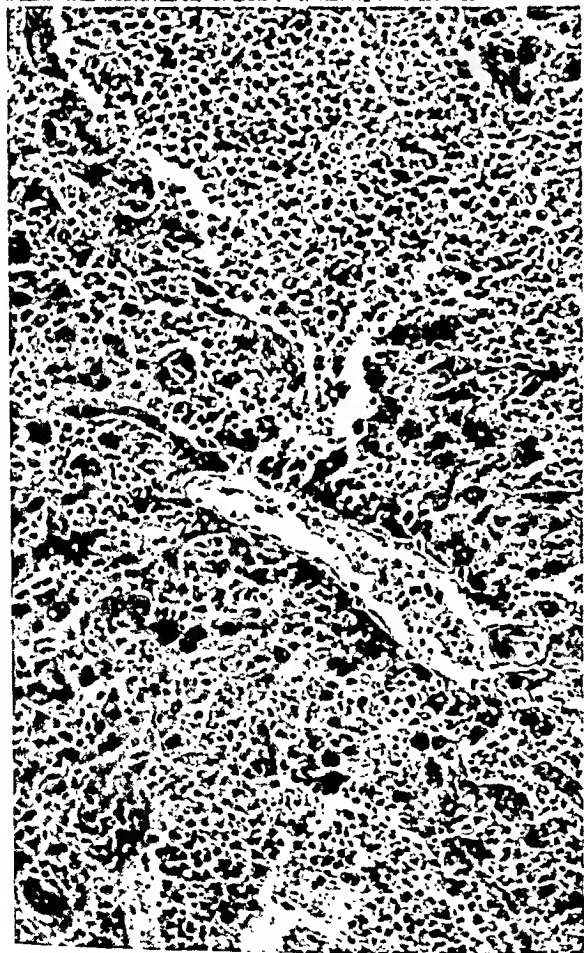
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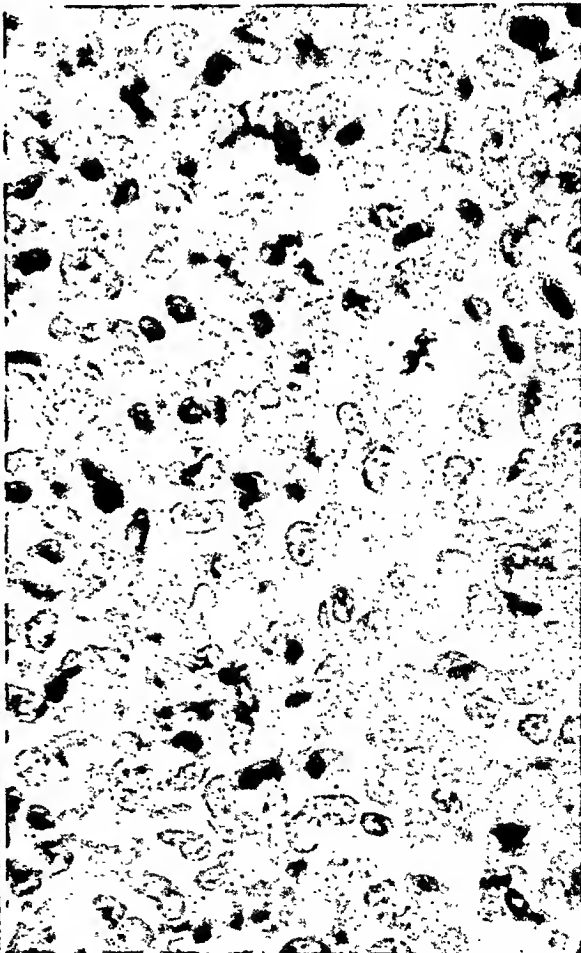
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9



10





# HEMORRHAGIC DIATHESIS EXPERIMENTALLY INDUCED BY DEFICIENCY IN VITAMIN K

## A HISTOPATHOLOGIC STUDY \*

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Since Dam's<sup>1,2</sup> studies on the sterol metabolism of chicks, which later on led to discovery of vitamin K (anti-clotting or anti-hemorrhagic vitamin), numerous articles dealing with the physiochemic, biochemic, physiologic, and therapeutic properties of vitamin K have been published.<sup>3-17</sup> However, the general pathology and especially the histopathology of vitamin K deficiency has been neglected.<sup>18-21</sup>

In a previous publication we reported the histopathologic findings in the central nervous system in the course of experimental vitamin K deficiencies in chicks and rats.<sup>22</sup> The purpose of the present paper is to present a detailed description of the general clinical observations and of the histologic changes in various tissues and viscera, and to discuss their relationship to other dietary deficiencies and to some similar findings in human pathology.

## EXPERIMENTAL MATERIAL

In this investigation, 120 white Leghorn chicks and 90 albino rats were used.

Both chicks and rats (first and second generations) were divided into two groups. In one group the acute type, in the other, the subchronic and the chronic type of deficiency was investigated. All animals were fed with the following basic, modified, Dam-Schönheyder's vitamin K-free diet: casein,† 20 per cent; yeast,‡ 15 per cent; sugar, 62.3 per cent; salt mixture,§ 2.7 per cent; vitamins A and D in the form of cod-liver oil (Schieffelin), 4 cc. to 100 gm. of diet; vitamin E in the

\* Received for publication, November 21, 1945.

† S.M.A. Corp. (Wyeth), vitamin-free casein as suggested by S. Ansbacher.

‡ Fleischmann's brewers' yeast containing the following vitamins: thiamine hydrochloride, 1-1.6 mg.; riboflavin, 4-6 mg.; niacin, 36-54 mg.; pyridoxine, 5-7.5 mg.; pantothenic acid, 12-18 mg.; and undetermined amounts of all other vitamins of B complex-natural to yeast.

§ Salt mixture no. 351 prepared as indicated by Hubbell, Mendel, and Wakeman.<sup>24</sup> In a second series of experiments the formula was as follows: calcium carbonate, 543 gm.; magnesium carbonate, 25 gm.; magnesium sulfate, 16 gm.; sodium chloride, 69 gm.; potassium chloride, 112 gm.; potassium phosphate (di-basic), 212 gm.; iron phosphate, 20.5 gm.; potassium iodide, 0.08 gm.; sodium fluoride, 1 gm.; aluminum potassium sulfate, 0.17 gm.; copper sulfate, 0.19 gm.; and MnSO<sub>4</sub> increased from 0.35 gm. to 0.5 gm. per cent (of total salt).

form of wheat germ oil (Sharpe and Dohme). Vitamin C was omitted because these animals can synthesize enough ascorbic acid to avoid scurvy.<sup>23</sup>

#### ACUTE VITAMIN K DEFICIENCY

##### *Chicks*

##### Clinical Observations

Two-day-old white Leghorn chicks were maintained on a normal diet and observed in laboratory condition for 2 or 3 days. Apparently normal, selected chicks were then put on Dam-Schönheyder's modified diet, as described above.

The first signs of deficiency appeared in approximately 7 to 14 days after the beginning of the experiment.\* Usually the hemorrhagic manifestations were preceded by some changes in the general appearance and behavior of the chicks. Some appeared hyperactive and somewhat restless in that they were irritable and had a tendency to scratch themselves, particularly around the neck, wings, thorax, tail, and abdomen. Close observation frequently revealed congestion of the superficial blood vessels, particularly around the knee joints and the ventral surface of the wings and toes. At the same time some of the chicks limped and had a tendency to sit down after taking only a few steps. They seemed fatigued and unable to maintain the body in an erect position. At times they tried to support themselves by leaning against the walls of the cage. In eating they often sat down. Concomitantly, more or less pronounced swelling of the knee joints with marked dilatation of the blood vessels (as revealed by transillumination) was frequently noticed. Fighting was quite often observed, after which bleeding sometimes persisted for several hours. At that stage, changes in the general aspect of the feathers and loss of feathers were noticed (Fig. 1).

Approximately after 8 to 15 days, more frequent and more marked hemorrhages were noticed. Their form, size, and diffusion varied from chick to chick. Although they seemed to appear spontaneously, we also had the impression that the hemorrhages easily followed very small and almost insignificant injuries. These were frequently aggravated by self-scratching or "picking." The hemorrhagic manifestations were more common around the roots of the feathers over the neck, breast, wings, thighs, and on the legs, toes, and toenails. Generally, bleeding was more pronounced in those regions which were more exposed to mechanical irritation or trauma. At times, the hemorrhages were localized in the legs, joints (Fig. 2), or wings (Fig. 3). At others, they were markedly diffuse and persisted until death. They were present more

\* We gained the impression that there was a seasonal influence in relation to the appearance and intensity of the hemorrhagic manifestations. They appeared more promptly and were more marked in February, March, and April.

frequently in the superficial tissues although observable also in the deeper tissues and the viscera. In many instances the stools appeared stained with blood. The benzidine test as well as the Meyer reaction for blood was intensively positive.

During the hemorrhagic stage some of the chicks disclosed hypermetria or dysmetria, noticeable in their gait, anterolateral pulsion or retropulsion, poor equilibrium in the erect position, and at times increased muscular tonus in the legs ("support reaction"). One chick presented marked unilateral exophthalmos due to a retrobulbar hematoma found at autopsy. Some gradually became unable to walk or to maintain an erect position. On some occasions chicks were seen lying on their sides with hyperextended extremities. In a few animals involuntary movements and abnormal posture of the head and neck were observed.

At this stage of the experiment death of some birds occurred almost suddenly. With others, death was preceded by an agonal stage, the duration of which varied from a few hours to a day. During this period irregular clonic-tonic or choreo-athetotic movements were noticed occasionally. In a few instances marked opisthotonus was present, somewhat similar to the posture described by Pappenheimer and associates<sup>58</sup> in chicks suffering from encephalomalacia.

The majority of chicks were sacrificed by ether, while some were autopsied immediately after spontaneous death. Tissues were fixed in 10 per cent alcohol, neutral formalin, formol-bromide, Zenker's, and Bouin's solutions. For microscopic investigation the following histologic methods were used: hematoxylin and eosin, Masson's trichrome stain, Weigert's stain for elastic tissue, and van Gieson's method for connective tissue. For the nervous system, Nissl's stain for nerve cells, Hortega's silver carbonate impregnation for microglia and oligodendroglia, Cajal's gold sublimate for astroglia, and Roizin's<sup>57a</sup> combined method for myelin sheaths and lipid products of disintegration were used. For the capillary network, Pickworth's<sup>57b</sup> and Eros' <sup>57c</sup> methods were applied.

### Autopsy Findings

Macroscopically, there was marked congestion, and small and large hemorrhages were noticed in the subcutaneous tissue, muscles (Figs. 4, 5, and 6), knee and shoulder joints, periosteum and bone, retroocular regions, lungs, gizzards (Fig. 7), intestines, liver (Fig. 8), kidney (Fig. 9), meninges, and brain.

The microscopic studies confirmed the occurrence of hemorrhagic manifestations in the skin and muscles (Fig. 10), joints and bones, esophagus, gizzard (Fig. 11), intestine (Fig. 12), liver (Fig. 13), sub-

capsular and parenchymatous regions of the kidneys (Figs. 14 and 15), heart (Fig. 16), lungs (Fig. 17), spleen, gonads, thymus, meninges, choroid plexus and ventricles, brain stem, cerebellum, and spinal cord.

In many instances, the hemorrhages were within the confines of the perivascular spaces forming an actual extramural hematoma; in others they were free in the parenchymatous tissue. The hemorrhages appeared as round, oval, or irregular, and varied greatly in size, from small capillary or precapillary areas to those that could be detected with the naked eye. Frequently they enveloped the blood vessels in "cuff-like" fashion. In some sections, no blood vessels could be discerned within the hemorrhagic area; in other areas, several blood vessels were included in it (Fig. 18). In some instances several hemorrhagic foci appeared, separated only by scanty and disorganized parenchyma (Fig. 19).

Some hemorrhages were so recent that relatively little degenerative change had occurred in the blood elements or in the involved tissues. In other areas the tissue of the involved organs was infiltrated with blood elements in various stages of disintegration. Numerous large and smaller mononuclear phagocytes containing brownish or yellowish amorphous granules or pigment were often found in these areas (Fig. 20). Some of these granular products gave a positive Perles' reaction for iron. In the brain tissue, phagocytic elements originating from microglial cells were often encountered.

Histologic studies revealed also various changes of the blood vessels. A majority of the arteries and veins were dilated; in some cases the capillaries appeared numerous and hyperemic (Fig. 21). Frequently, globular, ampullar, or irregular dilatations of the blood vessels and capillaries were noticed (Figs. 22, 23, and 24). In some instances the intimal cells of the arteries were swollen and pale, with the remainder of the wall edematous or thickened (Fig. 25). Both homogenization of the walls of vessels and discontinuity of some of the layers or rupture were noticed (Fig. 26).

The veins frequently appeared markedly distended, their walls irregular and often thinned. The intimal cells were round or ovoidal and at times pale or colorless, the muscle layer hardly discernible, and the adventitia poorly demarcated (Fig. 27). Although the vascular changes were generally seen in areas involved by hemorrhages, they were also encountered in the neighboring or even in far distant regions. Frequently, modifications of the content of the blood vessels were evidenced by a central conglomeration of the erythrocytes with peripheral displacement of the leukocytes (Fig. 28) with some diapedesis and perivascular edema.

### *Rats*

In the first group of experiments, 20 albino rats, weighing from 120 to 250 gm., were maintained on the same vitamin-deficient diet as used for chicks, for a period of approximately 10 months. Grossly, no definite hemorrhages were observed with the exception that some rats presented somewhat increased bleeding time and prolonged clotting time.

In a second group of 20 young rats put on the same vitamin K-deficient diet at the time of weaning (average weight, 38 to 45 gm.) from 7 to 12 days after the beginning of the experiment, hemorrhagic manifestations were noticed in the nails (Figs. 29 and 30) or around the toenails and the skin of the toes.

A third group of 15 adult, pregnant rats were put on the same vitamin K-deficient diet. Vitamin E (alpha-tocopherol, Merck) was, however, administered by injection to secure a normal pregnancy (as suggested by Pappenheimer *et al.*).<sup>58</sup> The offspring of these rats presented spontaneous hemorrhagic manifestations immediately or a few days after birth, which were identical to those described in the chicks, although somewhat less frequent and less diffuse. With the addition of from 5 to 10 per cent mineral oil<sup>25</sup> to the deficient diet, the bleeding appeared more marked and more diffuse. In these rats the hemorrhages were observed more frequently in those regions of the body exposed to mechanical irritation or traumatization, especially the tail, toes, nails, feet, legs (Figs. 31 and 32), abdomen, thorax, and occasionally the face; also, subcutaneous tissue and muscles (Fig. 33), knee joints, subarachnoid space (Fig. 34), retro-ocular region (Fig. 35), pelvic region (Fig. 36), some of the abdominal and thoracic viscera, brain, and cerebral ventricles (Fig. 37).

From a clinical point of view the rats did not exhibit the dysmetria, loss of equilibrium, and fatigability described in the chicks. However, some of the animals revealed limping or slight spastic paraparesis, which occurred mostly in the very young and was transient in character. One of the rats presented a marked bilateral and asymmetric exophthalmos which at autopsy was related to a retro-ocular hematoma. Another young rat developed generalized tonic-clonic convulsions a few hours before death. Autopsy revealed the presence of massive intracerebral and intraventricular hemorrhages.

Histologic studies of the various tissues and organs revealed changes of the same type and character as those described in the chicks, although generally less numerous and involving mostly the external tissues (such as skin, subcutaneous and muscular tissues).



## SUBCHRONIC AND CHRONIC VITAMIN K DEFICIENCY

The chicks and rats used for this investigation were maintained on the basic vitamin K-deficient diet until they revealed the first sure signs of vitamin K deficiency, *i.e.*, spontaneous hemorrhages and prolonged bleeding. At this point the animals were fed daily either the synthetic vitamin K (Proklot, Lilly \*) alone or with addition of biliary salts (Bilron, Lilly \*) until the initial external hemorrhagic manifestations completely disappeared. Some of these animals did not benefit from the addition of vitamin K (combined or not with Bilron), although the majority improved considerably or recovered completely. When recovery occurred (from 4 to 7 days), the animals were reinstated on the original vitamin K-deficient diet. A few animals spontaneously survived the acute episode without the addition of the synthetic vitamin or biliary salts. These animals were also included in this group.

*Chicks*

The chicks treated as above, *i.e.*, allowed to recover from the first acute episode, and then reinstated on the original vitamin K-deficient diet, survived for months up to 1½ years, when they were sacrificed for experimental needs. The majority developed well and, as far as curves of growth are concerned, showed no marked difference from a control group maintained on the synthetic vitamin-deficient diet to which vitamin K (Proklot) was added. However, some of the chicks reaching the adult age presented various alterations of the knee joints and bones which will be discussed in detail later.

A large number disclosed changes in the general aspect of the feathers (roughness and fragmentation). Also, loss of feathers occurred, usually in the regions more exposed to mechanical irritation (the anteroventral region of the thorax, and the abdominal regions), around the tail, and less frequently and in a lesser degree, on the legs and dorsal region of the body.

The hemorrhagic manifestations were much less frequently observed than in the acute phase of the experiment and they were more localized. When present, they generally appeared at the roots of the feathers or as small or larger hematomas located mostly in the subcutaneous tissue (Fig. 38). At times small and large hematomas were found at autopsy in the deeper tissues (Fig. 39), abdominal cavity, and various viscera (Fig. 40).

Small hematomas were reabsorbed or organized, but occasionally

\* These products were kindly supplied by Eli Lilly Co. to whom we wish to express our thanks.

they ruptured by traumatization or spontaneously, leading in some instances to the death of the animal (Fig. 41).

In post-mortem material three common alterations involving the different tissues and organs in various degrees were noticed: hemorrhages, vascular alterations, and reactive or degenerative changes directly or indirectly related to the other two.

The hemorrhages were either of recent character or older. The more recent were very similar to those described in the acute stage of vitamin K deficiency, although less frequent and less diffuse. The older ones could be divided into two types: hematomas more or less organized (Fig. 42), variable in size and in form; and more or less free, massive hemorrhages. Histologically, the latter appeared generally as hemorrhagic masses showing varying degrees of disintegration. In the majority the blood filled the interstitial spaces or replaced the destroyed structure of the organ. In the lung, for instance (Fig. 43), many of the alveoli were stuffed with blood and their outlines were no longer visible, owing to necrosis of the alveolar walls. The cellular details were lost and only shadowy network of the tissue remained to identify lung. Here and there large amounts of amorphous, golden brown or granular pigment, most of it intracellular within large mononuclear phagocytic cells, was observed. Some of these phagocytic elements containing iron pigment could be compared to the "dust cells" of anthracosis. In certain areas, extracellular pigment was also noticed where red cells had disintegrated in the interstitial tissue. Similar changes were noticed in the kidney (Fig. 44), spleen, brain (Fig. 45), and other tissues.

The blood vessels and the perivascular spaces of the various viscera and tissues, mostly when surrounded by blood or blood pigments, revealed differing pathologic changes. In some, the perivascular spaces appeared enlarged and contained, at times, various amounts of fatty products of degeneration (Fig. 46). In others, the surrounding tissue elements were also undergoing marked fatty degeneration (Fig. 47). In the central nervous tissue, particularly in the white matter of the brain, some of the blood vessels were surrounded by gitter cells containing various amounts of products of myelin disintegration besides hematic pigments, as well as progressive, reactive, and degenerative changes of the glial elements. Here and there perivascular demyelination also was observed (Fig. 48).

The vascular walls themselves were variously involved. In some the intimal cells were swollen, pale, and occasionally appeared thickened and hyperplastic. Even stratification of the endothelial cells was present (Fig. 49). The media, at times, showed degeneration of muscle fibers or increase in connective tissues, demonstrated by van Gieson's

method for connective tissue. Weigert's method for elastic tissue revealed some discontinuity and disintegration of the elastica and only occasional hyperplastic proliferation of the elastic fibers. In some instances the adventitia of the blood vessels was found thickened and marked proliferation of the connective tissue was noticed. Occasionally, in certain arteries, thickening of the entire walls was observed (Fig. 50). However, stasis and pre-stasis, as expressed by central conglomeration of erythrocytes, peripheral displacement and diapedesis of the white blood cells, as well as some vascular changes (edema and occasional rupture of the blood vessel walls) described in the acute phase of vitamin K deficiency, were also encountered at times in the subchronic and chronic group of vitamin K deficiency.

Parenchymatous histologic changes were present in the lungs, heart, liver, spleen, kidneys, central nervous system, muscles, testicles, thymus, and skin. These structural alterations, the description of which follows, were more or less closely related to the vascular pattern or to the hemorrhagic condition.

These parenchymatous structural changes appeared mostly as scattered areas of variable size and shape, undergoing degeneration and surrounded by more or less normal structures. These changes varied from slight cloudy degeneration to severe degeneration and even complete disintegration or disorganization of the basic histologic structures (Fig. 51). Here and there foci of necrosis were detected. Débris of the morphologic elements of the blood and hematic pigments were observed quite frequently in the same areas. Generally, disintegrated extravasated blood was free in the tissues but in some instances was in mononuclear phagocytes and macrophages.

In the liver, at times, striking distention of the sinusoids, especially in the center of the lobule, was seen. The sinusoids were then bordered by markedly compressed liver cords. For the most part the liver parenchyma showed degenerative changes only occasionally. However, in the tissues surrounding the blood vessels fat-staining methods revealed fatty products of degeneration which were frequently observed also in the blood vessel walls themselves.

In the nervous tissue, in addition to the already described morphologic alterations, occasional areas of demyelination, mainly surrounding blood vessels, were observed. There the combined method for myelin sheaths and lipids disclosed also the presence of lipidic products of degeneration (Fig. 52). In the brain and cerebellum scattered cortical areas disclosed nerve cell degeneration and acellular areas of varied dimensions. In the cerebellum small areas were found in which most of

the Purkinje and granular cells had disappeared with only remnants of cells in the acellular areas (Figs. 53 and 54).

In all affected viscera and tissues, scattered areas revealing more or less intense reparative processes often were observed. In these areas it was almost impossible to recognize the original structure of the organ replaced by proliferated connective tissue, leading at times to fibrosis (Figs. 55, 56, and 57) and scar-like formation or, in the brain, to glial hypertrophy and hyperplasia (Figs. 58 and 59).

### *Rats*

Rats were treated in the same manner as the chicks of the corresponding subchronic and chronic group. Generally speaking, one may say that in chronic vitamin K deficiency there were some slight differences between the results in the two groups: (a) In the chronic as well as in the acute stages, hemorrhages were more frequent in chicks than in rats; (b) In younger rats in the chronic state of deficiency, although hemorrhages were more frequent than in adults, this difference was less pronounced than in the chicks under the same experimental conditions. Furthermore, in rats of the second generation, the offspring of those maintained on a vitamin K-deficient diet to which vitamin E (alpha-tocopherol) was added during pregnancy, the frequency and the intensity of hemorrhages were greater than in rats born of normal parents; (c) Spontaneous recovery (from the acute deficiency) was more frequently noticed in the rats particularly after the weaning age. (d) Reabsorption of small hematomas was more frequent in the more mature or adult rats than in the chicks, although some localized and organized hematomas were noticeable as much as 14 months later (Fig. 60).

The histopathologic findings in this group of rats were similar to those described in the chicks in the subchronic and chronic stage with the exceptions that: (1) The hemorrhagic manifestations and secondary structural changes of the involved tissues were found mostly in the superficial tissues (particularly skin, subcutaneous tissue, and muscles); (2) These morphologic changes were much less frequently encountered than in the corresponding chick group.

One rat (second generation) kept on the vitamin K-deficient diet for 340 days gradually developed an apparent genito-lipodystrophy somewhat similar to Fröhlich's type. The animal weighed 480 gm., as compared with 350 gm. for a normal control. At autopsy a small organized hematoma in the retrosellar region of the skull was found (Fig. 61). Through compression it produced a marked depression at the

base of the brain involving the retrochiasmatic portion of the hypothalamus, the mammillary bodies, the fossa interpeduncularis, and a small part of the anterior portion of the pons (Fig. 62).

Some incidental histologic findings in chicks seemed to us the expression of complicating factors rather than of pure vitamin K deficiency.

(a) Marked proliferation of capillaries in the brain was observed in certain cases (Fig. 63a) and recalled somewhat similar vascular changes noted by us <sup>26</sup> in the cerebral cortex of cats in experimental inanition, and by Alexander <sup>27</sup> and Zimmerman and Burack <sup>28</sup> in selected areas in the central nervous system in vitamin B<sub>1</sub> deficiency.

(b) Gizzard erosions were found mostly in chicks in the acute stage and in a very few chicks in the subchronic stage of vitamin K deficiency.

(c) Small areas of encephalomalacia (Fig. 63b) were occasionally encountered. These areas were scattered in the cerebrum and cerebellum and consisted, especially in the latter, of degenerative changes of the parenchyma. Nerve cells, myelin sheaths, nerve fibers, and glial elements exhibited signs of advanced disintegration up to complete demyelination. Surrounding these areas reactive progressive changes of the glial elements were found. Similar areas of encephalomalacia were described in chicks by Pappenheimer and Goettsch <sup>29</sup> and Wolf and Pappenheimer, <sup>30</sup> and were considered by them to be the expression of vitamin E deficiency.

(d) On a few occasions, granulomatous formations with giant cells were noticed in the lungs. Some were fibrotic and calcified. Occasionally, marked calcification of the alveolar tissue independent of the granulomatous reaction also was observed.

(e) Another finding of interest from the standpoint of symptomatology in chicks was the occurrence in some of them of chondro-osteodystrophies in the subchronic and chronic group. This condition usually developed gradually. At first the chick experienced some difficulty in walking consisting chiefly in fatigue of the extremities and occasional limping. A few days later slight swelling of the knee joints was noticed which increased gradually in the subsequent days. Although this phenomenon more frequently involved only one side (Figs. 64 and 65), now and then bilateral swellings were observed. The chicks would then remain generally in a sitting position (even when eating or drinking). When stimulated to walk, they would support their bodies on the normal side, maintaining the affected extremity mostly in a flexed position. With the progress of time the involved extremity was less and less used while the flexor contraction would become more pro-

nounced and permanent. When the involvement was bilateral the chick became unable to walk (Fig. 66). At this stage slipping of the tendon from its proper position was frequently observed, as illustrated in anatomic preparations (Figs. 67, 68, and 69). In addition hemorrhages were observed in some of the affected joints and periarticular tissues. Roentgenologic examinations and gross and histologic studies revealed edema of the periarticular and articular tissues in addition to displacement, deformity, or dystrophy of various degrees involving the cartilages or the bony tissue, or both (Figs. 70, 71, and 72).

In the early stages of our investigation we felt that this condition was related to vitamin K deficiency. Later on an analysis of the diet pointed to a deficiency in manganese and that the condition was related to the so-called perosis or slipped tendon disease which was considered by Wilgus, Norris, and Heuser<sup>31,32</sup> and Inskso, Lyons, and Martin<sup>33</sup> as due to dietary manganese deficiency. To establish which part of the symptomatology was related to such deficiency, 20 white Leghorn chicks were put on the same vitamin K-deficient diet as used in the previous experiments with the exception that the quantity of  $\text{MnSO}_4$  of the salt mixture was increased from 0.35 to 0.5 gm. per cent (of total salt mixture). In this group we did not observe the typical slipped tendon condition. However, at times swelling of the knee joints, occasionally associated with abnormality of the posture or disturbed walking and some chondro-osteodystrophic changes, were still noticed although milder in intensity and much less frequent. Some of these alterations were considered by us as having definite relationship to the presence of hemorrhages or hematomas within the articulations (Figs. 73, 74, and 75) and in the bony tissue itself. This last occurrence in one case resulted in a fracture of bone (Fig. 76).

#### DISCUSSION AND COMMENTS

The opinion expressed in our previous report<sup>22</sup> on the histopathology of the central nervous tissue, concerning the appearance, frequency, and intensity of vitamin K-hemorrhagic diathesis, may be extended also to the other tissues and organs; *i.e.*, that in addition to the biochemical and physiopathologic effect proper to vitamin K deficiency, two main factors, age of the animals and action of mechanical irritation or external trauma, play an important rôle. In relation to the "age factor" we observed that the hemorrhagic diathesis was more marked and frequent in young chicks (7 to 12 days old) and in young rats (3 to 20 days old) than in adult animals which eventually become better adjusted to vitamin K deficiency.

The gross and microscopic changes noticed in the experimental

animals have a certain similarity to that which is known in human pathology as "bleeding disease of the newborn infants" or "morbus hemorrhagicus neonatorum." In fact, a large number of clinical investigations corroborate this similarity. In 1937, Brinkhous, Smith, and Warner <sup>34</sup> found that prothrombin in a bleeding newborn baby was exceedingly low and stated that the cause of the bleeding was hypoprothrombinemia. Later on Quick and Grossman <sup>35</sup> found that in all newborn infants there is a definite phase of physiologic hypoprothrombinemia from the second to the fifth day of life. Meanwhile Salomonsen <sup>36</sup> and Grossman <sup>37</sup> found that cerebral bleeding revealed itself precisely between the second and the third day of life. Further, Dam, Tage-Hansen, and Plum, <sup>38</sup> Nygaard, <sup>39</sup> and Quick <sup>40</sup> concluded that the physiologic hypoprothrombinemia is due to a dietary lack of vitamin K in the first few days of life and that subsequent establishment of the bacterial flora in the baby's intestines initiated the synthesis of vitamin K or similar antihemorrhagic substances which became an important factor in the spontaneous restoration of the normal prothrombin level.

As far as the action of mechanical irritation or external trauma is concerned, we observed particularly in both young chicks and young rats that the lesions were more pronounced in those regions of the body that were more exposed to mechanical irritation or external trauma. External hemorrhages appeared more frequently and were more pronounced when many animals were kept in the same cage making them more subject to fighting and to trauma. While from 60 to 70 per cent of the animals (mostly young, and particularly chicks) exhibited a cutaneous hemorrhagic diathesis, only 20 to 26 per cent presented visceral hemorrhages and even less (3 to 5 per cent) presented hemorrhages in the central nervous tissue. These figures seem to be related to the better protection of the viscera and especially the central nervous tissues against mechanical irritation or external trauma. In bleeding disease of the newborn human, it has also been found that trauma, and particularly the application of forceps, was a precipitating factor. Besides, Snedeker <sup>41</sup> reported that during the periods of physiologic hypoprothrombinemia minor operations such as circumcision or cutting of the frenulum of the tongue could be followed by unremitting bleeding which occasionally led to exsanguination.

Cattle and other animals, particularly ruminants, fed spoiled sweet clover hay develop a hemorrhagic diathesis characterized by prolonged clotting time and hypoprothrombinemia. <sup>42</sup> These animals often carry the disease in a latent form, but a slight trauma may bring about severe hemorrhages which may frequently terminate fatally (Rod-

erick<sup>43</sup>). However, it was demonstrated that hypoprothrombinemia in these animals is not due to vitamin K deficiency but to the reaction of a toxic substance, dicoumarin.<sup>44-46</sup>

Although age and trauma play a precipitating rôle in the causation of hemorrhages, the genesis of the hemorrhagic diathesis in the course of vitamin K deficiency lies fundamentally in the general or local biochemical and pathophysiologic changes which, according to the majority of authors, result from the reduction of prothrombin in the blood of both man and experimental animals. Through studies of blood clotting time it has been established that hypoprothrombinemia may be due either to the lack or insufficiency of vitamin K in the diet, or to its disturbed metabolism related to hepatic dysfunction or disturbed internal absorption. That the absence of vitamin K from the diet, in our experimental group of animals, is responsible for the genesis of hemorrhages was established not only by the fact that hemorrhages appeared only in animals fed with the Dam-Schönheyder modified diet, but also by the cessation of visible hemorrhages and recovery of animals following administration of synthetic vitamin K (Proklot, Lilly).

It has been reported by Elliott and his co-workers<sup>25</sup> that the addition of mineral oil to the diet causes hypoprothrombinemia by preventing absorption of vitamin K or by producing injuries to the liver. In our group of animals in which mineral oil was added to the diet the hemorrhagic diathesis appeared sooner and was more severe, thus supporting the hypothesis that mineral oil adds to the deficiency either by interfering with the assimilation of whatever trace of vitamin K may be present or inhibiting the intestinal fermentation (through which synthesis of vitamin K or similar anti-hemorrhagic substances may originate).

Our experimental data find their counterpart in human pathology. Recently it has been established that the tendency toward bleeding in intestinal disorders,<sup>47-49</sup> obstructive jaundice,<sup>50</sup> biliary fistula,<sup>51</sup> and liver diseases<sup>52, 53</sup> is related to an altered process of absorption or to defective metabolism of the anti-hemorrhagic factor rather than to a primary dietary deficiency of vitamin K. Because of this supposed relation between liver function and the metabolism of vitamin K, it has been suggested that bile salts be administered in addition to vitamin K to prevent the symptoms caused by such a deficiency. Guided by this suggestion, we have made use of biliary salts in our experimental work in order to secure more prompt action of vitamin K in preventing the early hemorrhagic manifestations in those animals in which recovery was attempted before resumption of the subchronic experiments. In addition to the action of precipitating factors such as age and



trauma, we feel that our histologic studies furnish some additional information concerning the physiopathologic mechanism of the hemorrhage itself. It is known that retardation of the blood current, through pre-stasis and stasis, is considered a factor predisposing to diapedesis of hemal elements. In addition, Landis<sup>54</sup> and Baron and Chambers<sup>55</sup> stated that stasis and typical concentration of red cells in the central part of blood vessels, as described by Krogh,<sup>56</sup> can result also from local mechanical injuries to blood vessel walls themselves without changes in diameter. Pickworth<sup>57b</sup> believed that central concentration of red blood cells and peripheral displacement of the white cells in the blood vessels are in relation to hydrodynamic changes in the circulation of the blood. Figures 27 and 28 illustrate the abnormal disposition of the white and red blood cells as well as a stage of their migration. We feel that vasodilatation and the marked congestion of the blood vessels occurring in the early stage of deficiency may play a part in the production of the hemorrhage.

Possibly the lack or deficiency of prothrombin may determine some disturbance of the hydrodynamics of the blood in addition to physicochemical or morphologic changes of the vessel walls which would facilitate diapedesis.

We would like to discuss briefly certain previously listed pathologic findings in chicks on the vitamin K-deficient diet, which require further investigation.

(a) In some areas of the brain marked proliferation of capillaries was noticed in a few chicks in the subchronic and chronic stages of vitamin K deficiency. This histopathologic finding recalled somewhat similar vascular changes described by us in the brain and brain stem of cats in experimental inanition<sup>26</sup> and by Alexander (pigeons)<sup>27</sup> and Zimmerman and Burack (dogs)<sup>28</sup> in the central nervous system in vitamin B deficiency. This may be related to other factors than simple vitamin K deficiency, such as general malnutrition or disturbed assimilation of vitamins B and B-complex.

(b) The presence of "gizzard erosions" was noticed in some of the chicks with hemorrhagic diathesis of the gizzard itself. On the other hand, many chicks with no appreciable hemorrhages in the gizzard revealed in the latter very severe erosions. In this regard our findings are in full agreement with those of Dam and Schönheyder,<sup>20</sup> Holst and Halbrook,<sup>19</sup> and Almquist and Stokstad.<sup>4</sup> We also agree with their opinion that gizzard erosions are not a characteristic symptom of vitamin K deficiency and that they may be initiated, to some extent, by traumatic stimuli.

(c) In a few chicks only, scattered small areas of encephalomalacia

in the brain and cerebellum were found. These areas are somewhat similar to those described in chicks by Pappenheimer and Goettsch<sup>29</sup> and later by Wolf and Pappenheimer<sup>30</sup> and considered by them and other workers as the expression of vitamin E deficiency.<sup>58-60</sup> We feel that in our animals there was no deficiency in vitamin E. Nevertheless, deficiency of vitamin E may originate in disturbed metabolism of other vitamins or nutritional factors. Such inter-relationship, if it exists, must be left for further investigation; especially in connection with the presence in the diet of various excessive fatty acids (Dam<sup>60</sup>).

(d) In the lungs of a few chicks in the subchronic and chronic stages of vitamin K deficiency granulomatous formations with giant cells were found. Some of them were fibrotic and calcified. No direct relationship with hemorrhages was detected. It seems that these histopathologic changes might be considered as a result of a complicating factor, such as secondary infections, rather than the direct effect of the vitamin K deficiency.

(e) The rôle of manganese in the prevention of perosis or slipped tendon disease was indicated by the observations of Wilgus *et al.*,<sup>31,32</sup> Gallup and Norris,<sup>61</sup> and Inskso *et al.*<sup>33</sup> Later on, another group of authors (Hogan and Richardson,<sup>62</sup> Jukes,<sup>63,64</sup> Hegsted, Mills, Elvehjem, and Hart,<sup>65</sup> reported that perosis, which occurred in spite of adequate dietary manganese, was prevented by constituents of an alcoholic extract of liver (Hogan *et al.*<sup>62</sup>), choline (Jukes,<sup>63,64</sup> Jukes and Welch<sup>66</sup>) and possibly by certain unidentified factors (Jukes,<sup>64</sup> Hogan *et al.*<sup>62</sup>). In our experiments we observed that the addition of a larger quantity of manganese to the vitamin K-deficient diet prevented only some of the changes related to slipped tendon disease or perosis, thus leaving the impression that a portion of this chondrodystrophy was related to the hemorrhages (Figs. 74, 75, and 76). On this point our findings are somewhat similar to the orthopedic findings of König<sup>67</sup> in 98 cases of hemophilia. He described: (1) "hemarthrosis" or the stage of the first hemorrhages into the joints; (2) "panarthrititis" or the stage of inflammatory reaction with changes in the bones and cartilages; (3) "regression" or the stage of permanent joint changes with erosion and contractures (Figs. 75 and 77).

Once hemorrhages have occurred in the joints, they exercise harmful effects, partially through a mechanical factor (pressure atrophy, etc.) and partially through inflammatory secondary changes of the synovial membrane, an opinion already expressed by König<sup>67</sup> and Fonio.<sup>68</sup>

We believe that trauma plays a very important rôle as a precipitating and aggravating factor in the pathogenesis of some of the chondrodystrophic changes. This is illustrated by the fact that the joints in-

volved are generally the knees (Figs. 65, 66, 67, 74, and 75), which the chicks used as the main support whenever unable to walk normally. Such continuous mechanical irritation leads to various secondary alterations and deformities in the joints and bones (Figs. 71, 72, and 73). Our observations agree with those of Fonio,<sup>68</sup> who reported that in cases of hemophilia the joints commonly involved are those most frequently exposed to trauma; namely the knee, the elbow, and the ankle.

### SUMMARY

White Leghorn chicks and albino rats (first and second generation) in acute, subchronic, and chronic vitamin K deficiency revealed various pathologic findings:

1. Hemorrhagic diathesis. This condition was characterized by hemorrhages in the skin, subcutaneous tissue, joints, muscles, lungs, heart, gizzard, liver, kidneys, spleen, bones, thymus, gonads, brain, spinal cord, meninges, choroid plexus, subarachnoid spaces, and cerebral tissue. Frequently the hemorrhages were within the confines of the perivascular spaces and enveloped the blood vessels in "cuff-like" fashion; at other times they were free in the parenchyma. The hemorrhages appeared round, oval, or irregular and varied greatly in size, from small capillary or precapillary to large hemorrhages easily detected with the naked eye. Often the hemorrhages appeared as scattered foci, some were separated only by scanty and disorganized interstitial or parenchymatous tissue, and others showed confluence of various foci. The hemorrhages were recent or older, depending upon the time of observation. At times they were more or less organized hematomas, or large phagocytic elements containing hematic granular pigment represented the only trace of previous hemorrhages. Recent hemorrhages were noticed more frequently in the acute stage, whereas hematomas and phagocytes containing hematic pigments were encountered more frequently in the subchronic and chronic stage.

2. Blood vessel changes. Frequently the blood vessels (veins and arteries) appeared markedly and irregularly dilated and congested. Here and there their walls appeared variously involved, from simple swelling and mild degeneration to complete rupture. These vascular changes were observed more frequently near or within the hemorrhagic areas. In some sections no blood vessels could be discerned within the hemorrhagic infiltrated tissue. Hypertrophy and hyperplasia of the blood vessel walls were also encountered. The latter was more often observed in the subchronic and chronic stage of deficiency.

3. Hydrodynamic disorders of the circulation as expressed by pre-stasic and stasic conditions were noted in the acute and subchronic stages, more often in the former.

4. Degenerative and reparative structural changes of different tissues and viscera, mostly secondary to the hemorrhagic conditions, were observed in both subchronic and chronic stages.

5. Proliferation of capillaries in certain areas of the brain, gizzard erosions, areas of encephalomalacia, granulomatous formations, and perosis or slipped tendon disease were noticed only in chicks. The second and third were observed more frequently in the acute and subchronic stage, the other two mostly in the subchronic and chronic stage. These changes were considered the expression of complicating factors. Determination of their relationship to vitamin K deficiency will depend on further experimentation.

The following factors played a rôle in experimental hemorrhagic diathesis in vitamin K deficiency:

1. Altered physicochemical properties of the blood, presumably hypoprothrombinemia, which may interfere with the proper hydrodynamics of the circulation of the blood and result in vasodilatation, stasis, diapedesis, and structural damage of the blood vessel walls.

2. Age of the animals, since hemorrhages were more severe and more prompt to appear in newborn or young animals.

3. Traumatic factors seem to play a definite precipitating rôle.

4. Dysfunction in the absorption (especially when mineral oil is added to the diet) and metabolism of vitamin K and possible hepatic dysfunction.

Our findings are similar to those in the bleeding disease of the newborn infant (morbus hemorrhagicus neonatorum), a condition which is also aggravated by trauma.

Gizzard erosions and encephalomalacia of chicks were not considered characteristic of the vitamin K-deficient diet, presumably being related to other nutritional factors still to be investigated.

The chondrodystrophies observed mostly in chicks in the chronic stage seemed to be partly related to perosis or slipped tendon disease and partly to hemarthrosis. The latter was considered as the expression of the hemorrhagic diathesis. Trauma seemed also to be an important factor in determining and aggravating the chondrodystrophies.

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## DESCRIPTION OF PLATES

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### PLATE 213

FIG. 1. Changes in the general aspect of the feathers and falling of feathers in chicks in acute vitamin K deficiency.

FIG. 2. Hemorrhages in a knee joint of a chick in acute vitamin K deficiency.

FIG. 3. Hemorrhagic suffusion in the subcutaneous tissue of a wing in a chick in acute vitamin K deficiency.



Ferraro and Roizin

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PLATE 214

- FIG. 4. Subcutaneous hemorrhagic blood clots, particularly pronounced in the antero-external surface of the leg of a chick in the acute stage of vitamin K deficiency.
- FIG. 5. Diffuse infiltrating hemorrhages in the muscles of the leg of a chick sacrificed in the acute stage of vitamin K deficiency.
- FIG. 6. Severe infiltrating hemorrhage in the pectoral muscle of a dead chick in the acute stage of vitamin K deficiency.



PLATE 215

- FIG. 7. Erosions (a) and hemorrhages (b) of various degrees and extension in gizzards in the acute stage of vitamin K deficiency.
- FIG. 8. Infiltrating hemorrhage in the liver of a chick sacrificed in the acute stage of vitamin K deficiency.
- FIG. 9. Infiltrating hemorrhages in the kidney of a chick sacrificed in the acute stage of vitamin K deficiency.
- FIG. 10. Histologic appearance of infiltrating hemorrhages of the skin, subcutaneous tissue, and subjacent muscles of a chick in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain.  $\times 34$ .
- FIG. 11. Histologic appearance of hemorrhages involving the superficial layers of the gizzard in a chick in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain.  $\times 20$ .

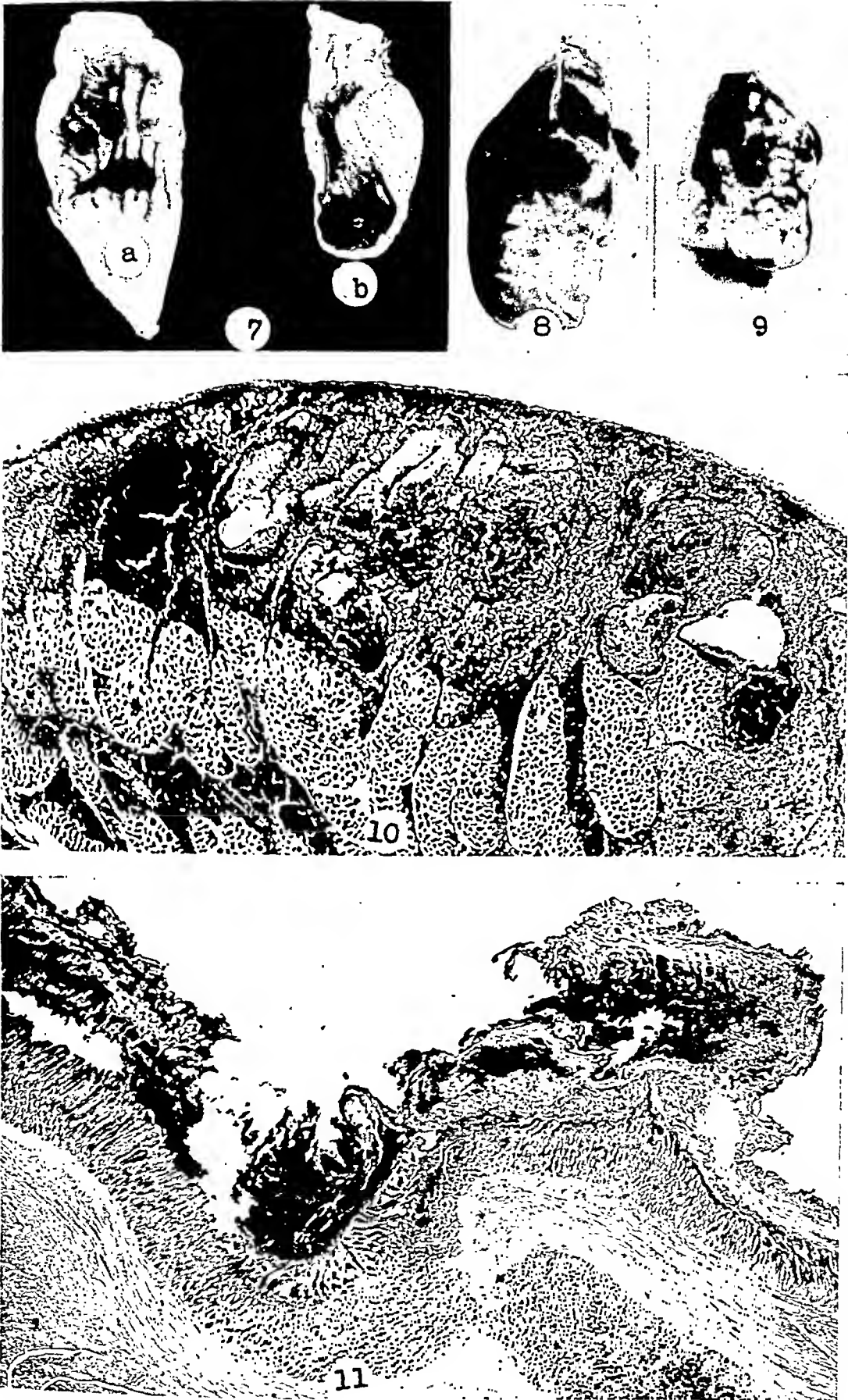


PLATE 216

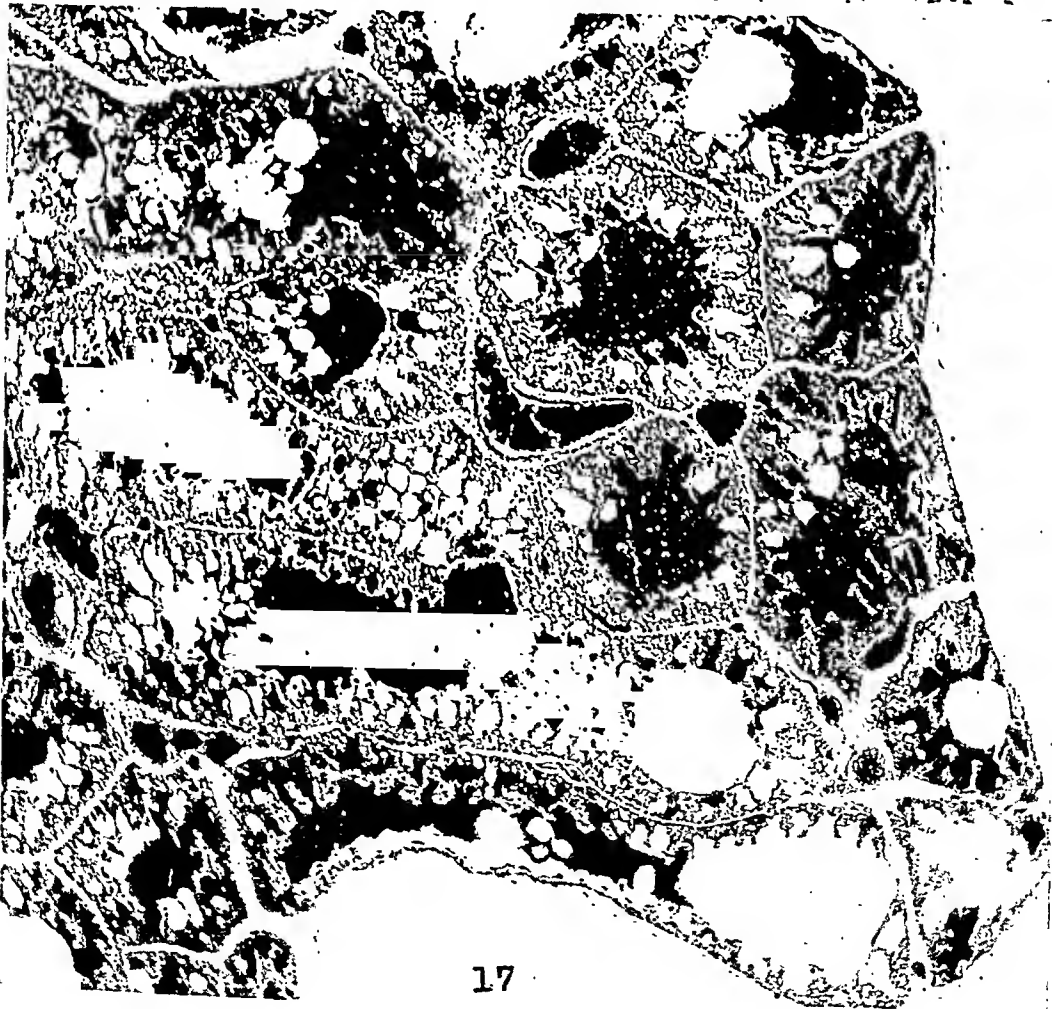
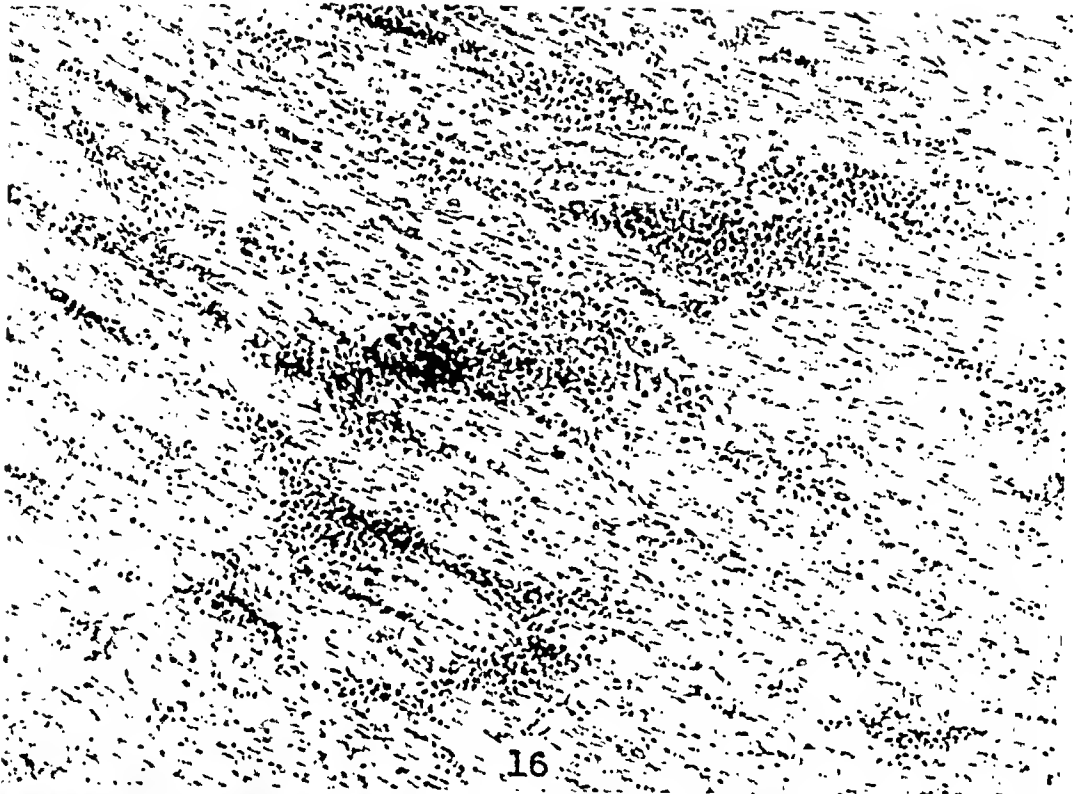
- FIG. 12. Hemorrhages involving mucosa, submucosa, and muscular layers of the small intestine of a chick in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain.  $\times 100$ .
- FIG. 13. Hemorrhages of varying size and form in the liver of a chick in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain.  $\times 200$ .
- FIG. 14. Histologic appearance of a massive subcapsular hemorrhage in the kidney of a chick in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain.  $\times 52$ .
- FIG. 15. Hemorrhages mainly involving the tubular structures of the kidney of a chick in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain.  $\times 100$ .



PLATE 217

FIG. 16. Hemorrhages in the myocardium of a chick in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain.  $\times 200$ .

FIG. 17. Small and large hemorrhages variously involving the pulmonary tissue of a chick in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain.  $\times 52$ .



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PLATE 218

FIG. 18. Central hemorrhage and congested radiating blood vessels outlined by diapedesis in the brain stem of a chick in the acute stage of vitamin K deficiency. Nissl's stain.  $\times 100$ .

FIG. 19. Diffuse hemorrhages leading to severe disintegration and disorganization of liver structure in a chick in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain.  $\times 140$ .

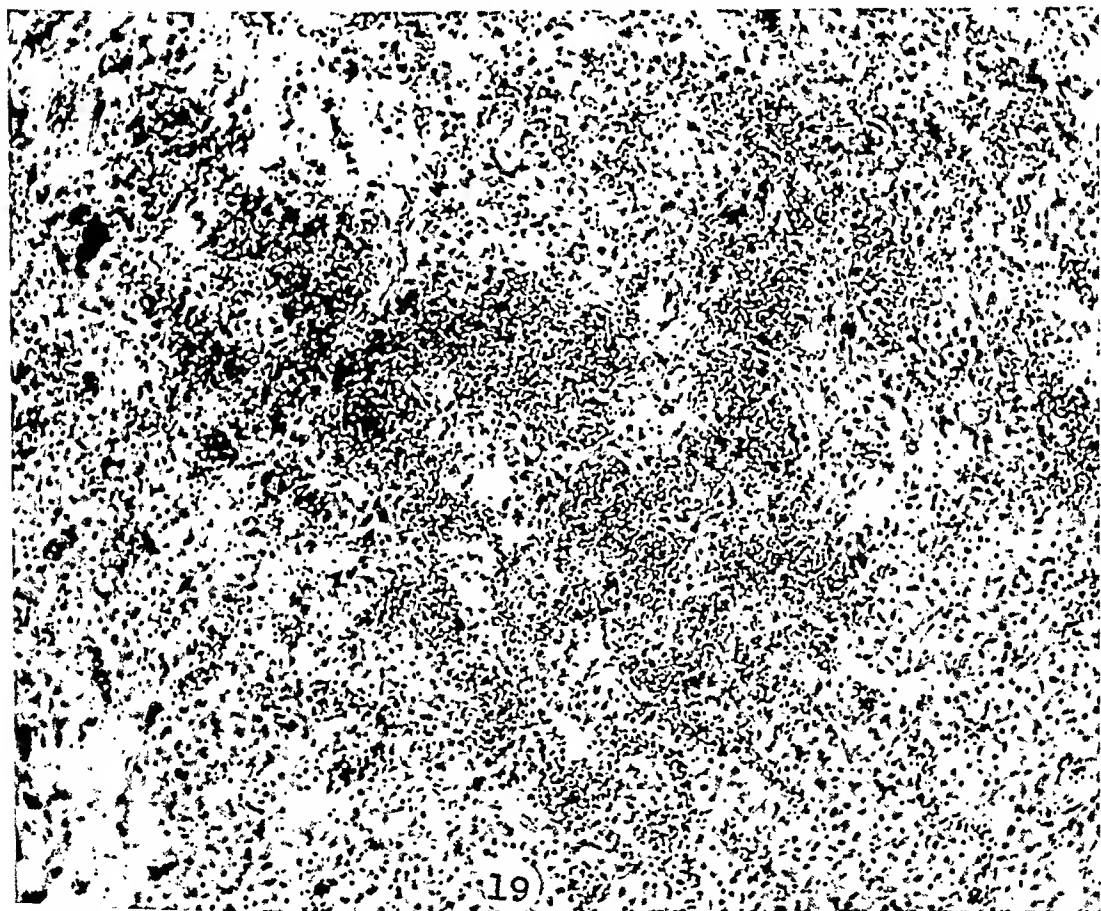
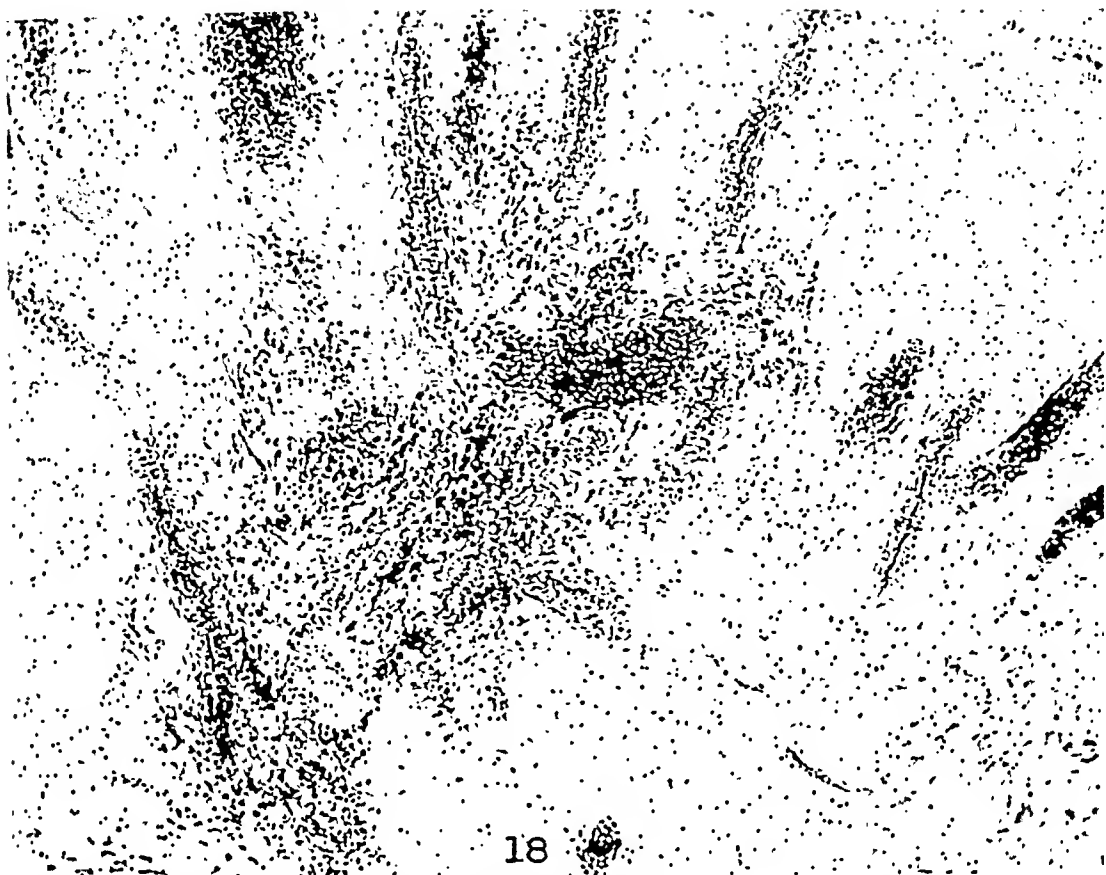


PLATE 219

FIG. 20. Large and small mononuclear phagocytic elements containing hemal granular material in the lung of a chick which died in the subacute stage of vitamin K deficiency. Hematoxylin and eosin stain.  $\times 560$ .

FIG. 21. Diffuse and marked vascular dilatation and hyperemia in the kidney of a chick sacrificed in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain.  $\times 34$ .

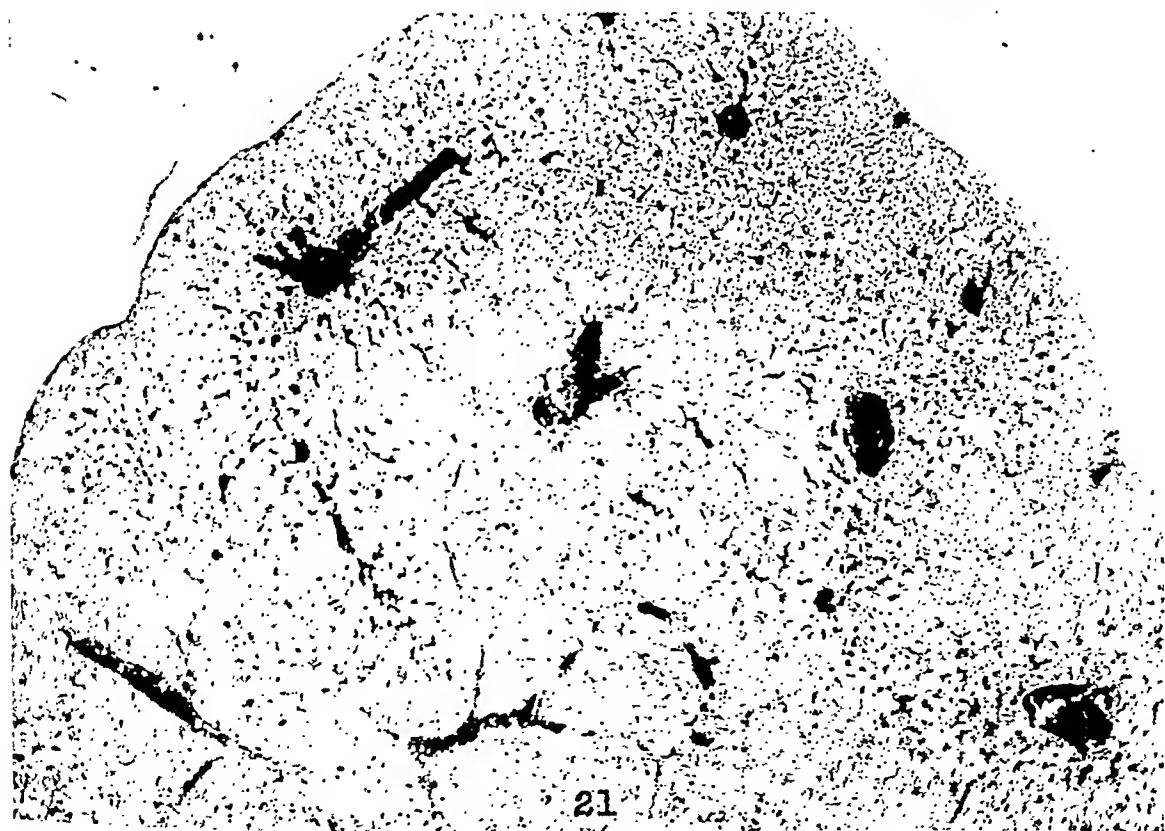
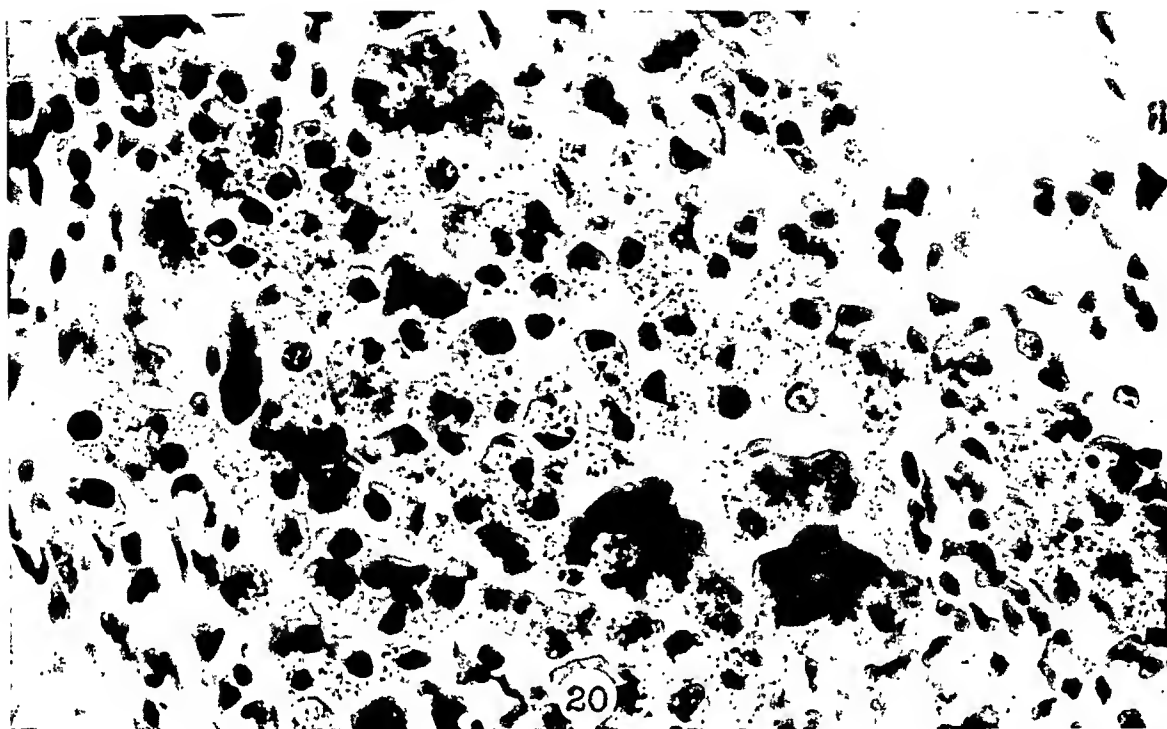


PLATE 220

- FIG. 22. Ampullar, globular, and irregular dilatation of blood vessels and capillaries in the cerebellar white matter of a chick in the acute stage of vitamin K deficiency. Nissl's stain.  $\times 52$ .
- FIG. 23. Small, circumscribed hemorrhages in the pons of a chick in the acute stage of vitamin K deficiency. Pickworth's method.<sup>57b</sup>  $\times 52$ .
- FIG. 24. Capillary and precapillary hemorrhages in the medulla of a chick, with tendency to diffusion, in the acute stage of vitamin K deficiency. Eros' method.<sup>57c</sup>  $\times 52$ .

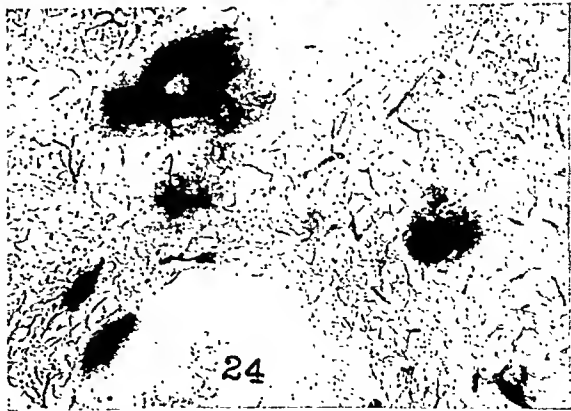
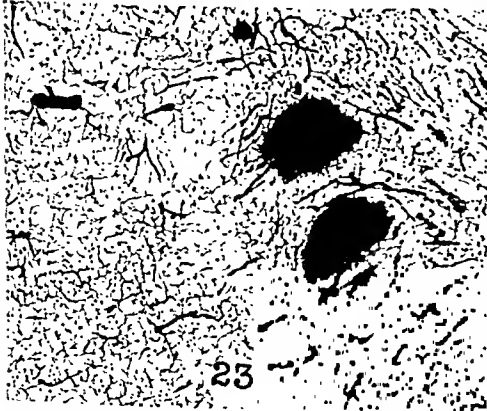


PLATE 221

- FIG. 25. Edema and thickening of the walls of a myocardial artery of a chick in the acute stage of vitamin K deficiency. Hematoxylin and eosin stain.  $\times 200$ .
- FIG. 26. Rupture of the walls of a cerebral artery in a chick in the acute stage of vitamin K deficiency. Weigert's elastic tissue stain.  $\times 200$ .
- FIG. 27. Perivascular edema and dilatation of a cerebral vein with diapedesis of blood elements in a chick in the acute stage of vitamin K deficiency.  $\times 200$ .
- FIG. 28. Central conglomeration of erythrocytes with peripheral displacement of leukocytes and tendency to diapedesis in a cerebral blood vessel of a chick in the acute stage of vitamin K deficiency. Nissl's stain.  $\times 200$ .

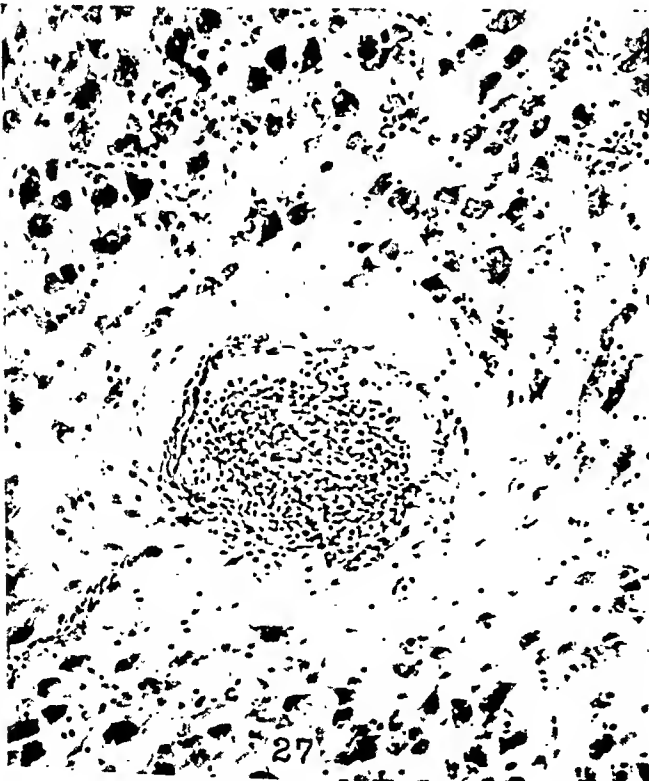
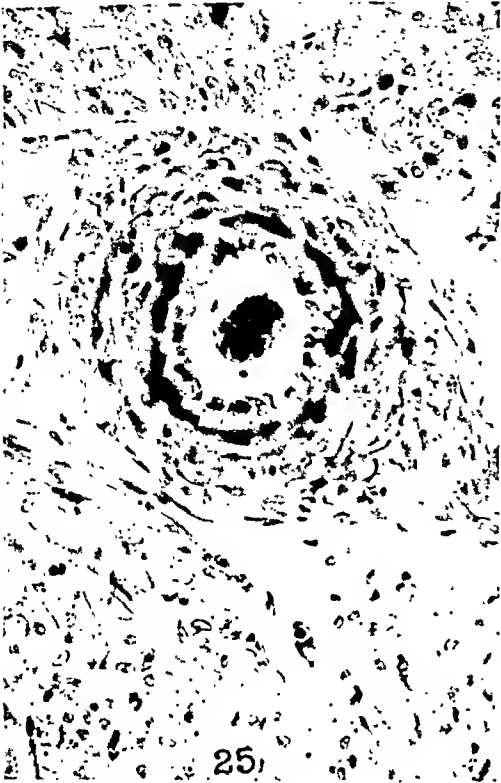


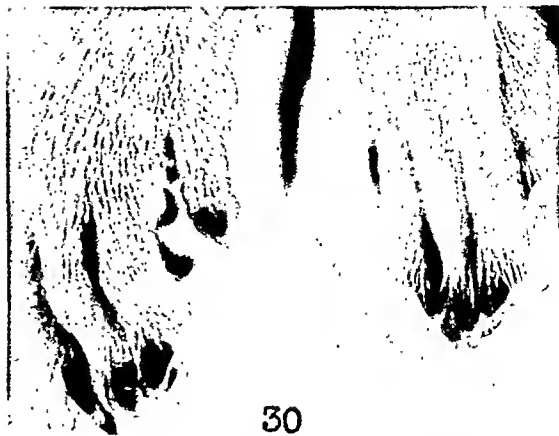


PLATE 222

- FIG. 29. Hemorrhages in the nails of the anterior extremities of a young rat in the acute stage of vitamin K deficiency.
- FIG. 30. Hemorrhages in the nails of the posterior extremities of a young rat in the acute stage of vitamin K deficiency.
- FIG. 31. Massive hemorrhages of the tail, toes, and nails of a young rat in the acute stage of vitamin K deficiency.
- FIG. 32. Hemorrhages in the knee joints, toes, nails, and tail of a young rat in the acute stage of vitamin K deficiency.
- FIG. 33. Massive hemorrhages in the subcutaneous and muscular tissues of the thorax of a young rat sacrificed in the acute stage of vitamin K deficiency.
- FIG. 34. Hemorrhages in the subarachnoid space, toe, and tail of a young rat which died in the acute stage of vitamin K deficiency.



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PLATE 223

FIG. 35. Massive hemorrhages in the retro-ocular region and region of the frontal sinus of a young rat which died in the acute stage of vitamin K deficiency.

FIG. 36. Pelvic hemorrhage in a young rat sacrificed in the acute stage of vitamin K deficiency.

FIG. 37. Intraventricular and parenchymatous cerebral hemorrhages in a rat in the acute stage of vitamin K deficiency. A few hours before death the animal developed generalized tonic-clonic convulsions.

FIG. 38. Ruptured large hematoma in the subcutaneous tissue of the ventral part of the left wing of a chick in the subchronic stage of vitamin K deficiency.

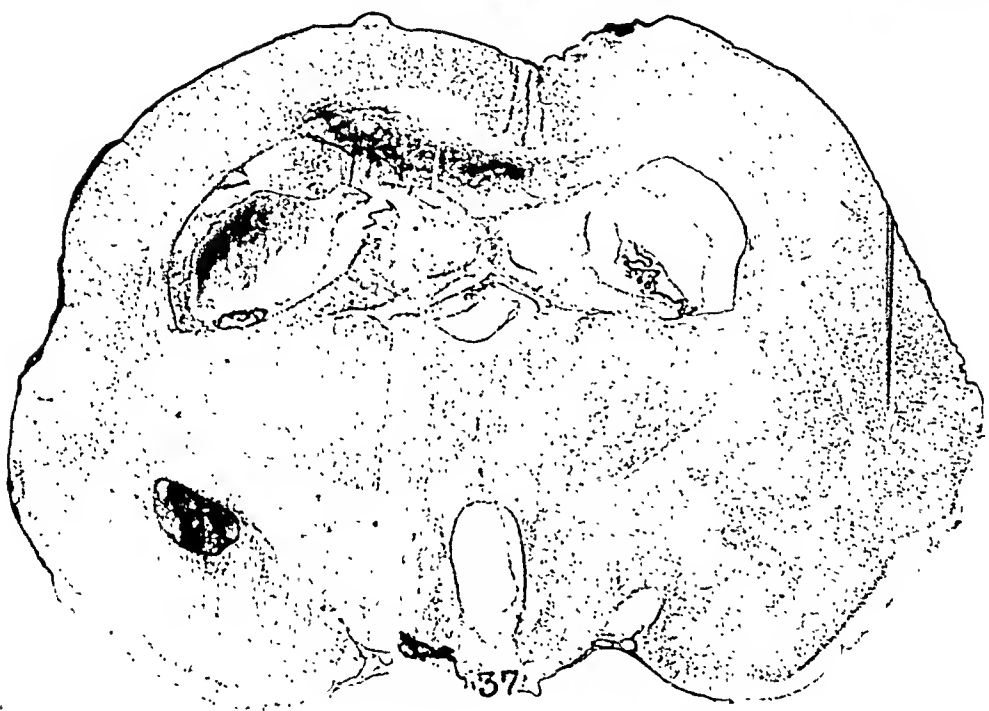
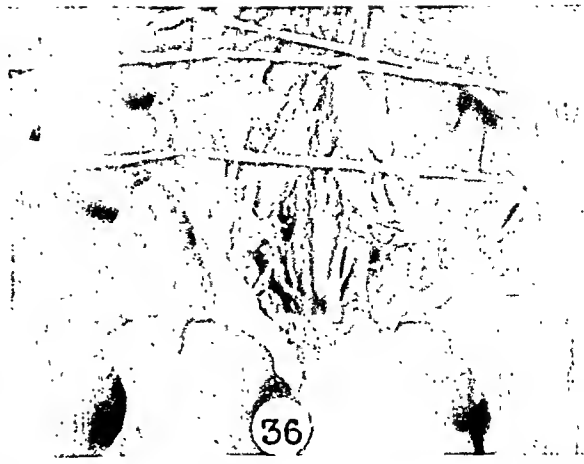


PLATE 224

FIG. 39. Intradural hematoma in the cervical region of the spinal cord of a chick sacrificed in the subchronic stage of vitamin K deficiency. Nissl's stain.  $\times 280$ .

FIG. 40. Large, partially organized hematoma in the myocardium of a chick sacrificed in the subchronic stage of vitamin K deficiency. Hematoxylin and eosin stain.  $\times 140$ .



PLATE 225

FIG. 41. Fatal, massive hemorrhage in the abdominal cavity of a chick in the sub-chronic stage of vitamin K deficiency.

FIG. 42. Small, organized hematoma in the inner muscular layer of the gizzard of a chick in the chronic stage of vitamin K deficiency. Hematoxylin and eosin stain.  $\times 100$ .

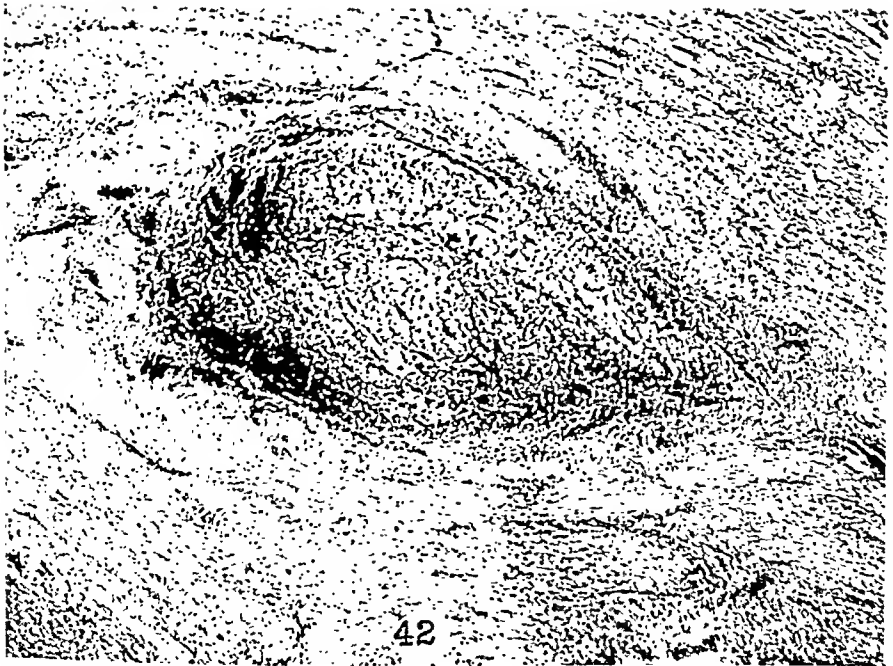




PLATE 226

FIG. 43. Diffuse, long-standing hemorrhage infiltrating and disintegrating the lung tissue of a chick in the chronic stage of vitamin K deficiency. Hematoxylin and eosin stain.  $\times 52$ .

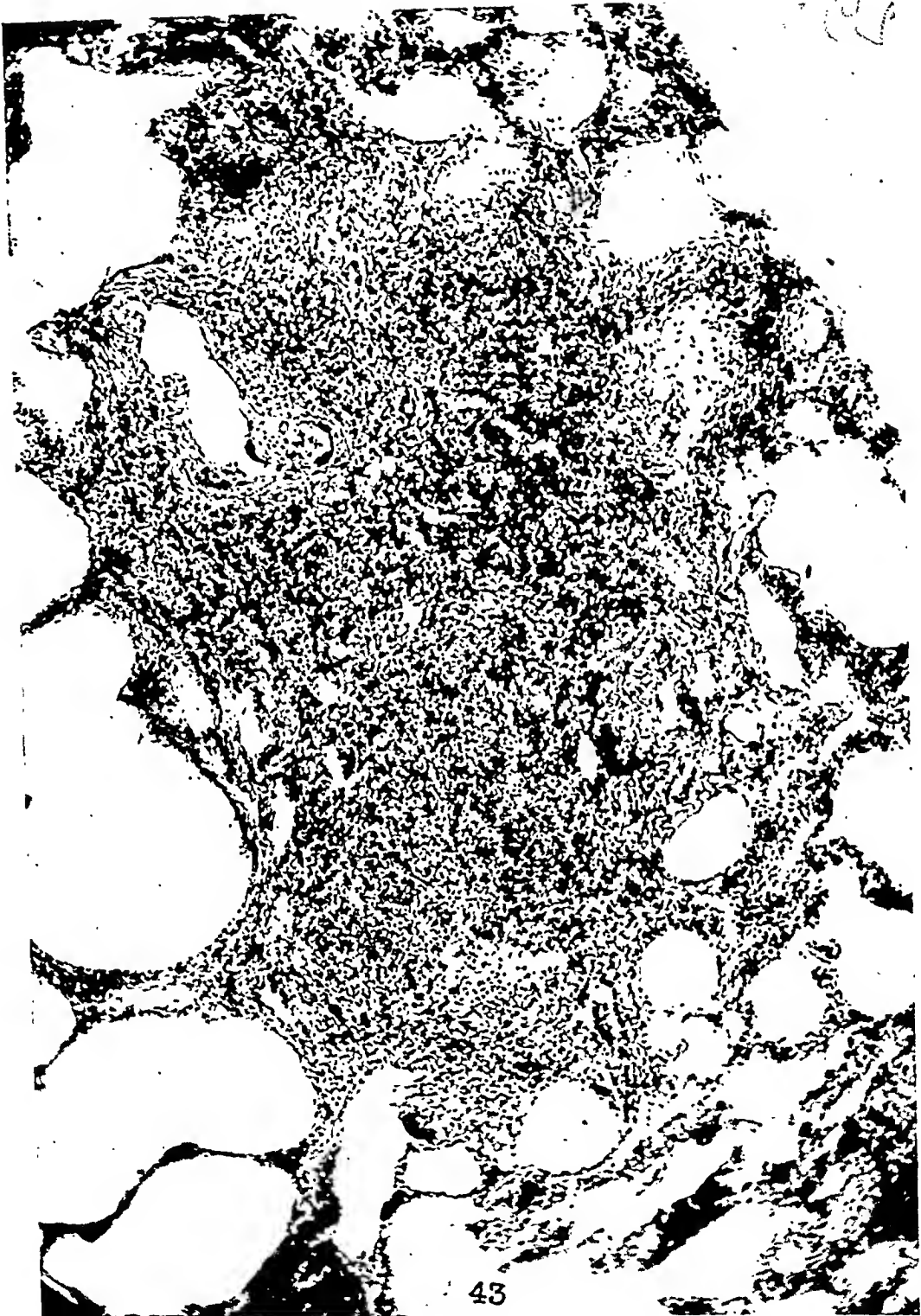


PLATE 227

FIG. 44. Amorphous granular pigment, mostly intracellular, in long-standing hemorrhage of the kidney in a chick in the chronic stage of vitamin K deficiency. Hematoxylin and eosin stain.  $\times 720$ .

FIG. 45. Compound granular corpuscles containing siderophile pigments. Chick brain in the chronic stage of vitamin K deficiency. Nissl's stain.  $\times 720$ .

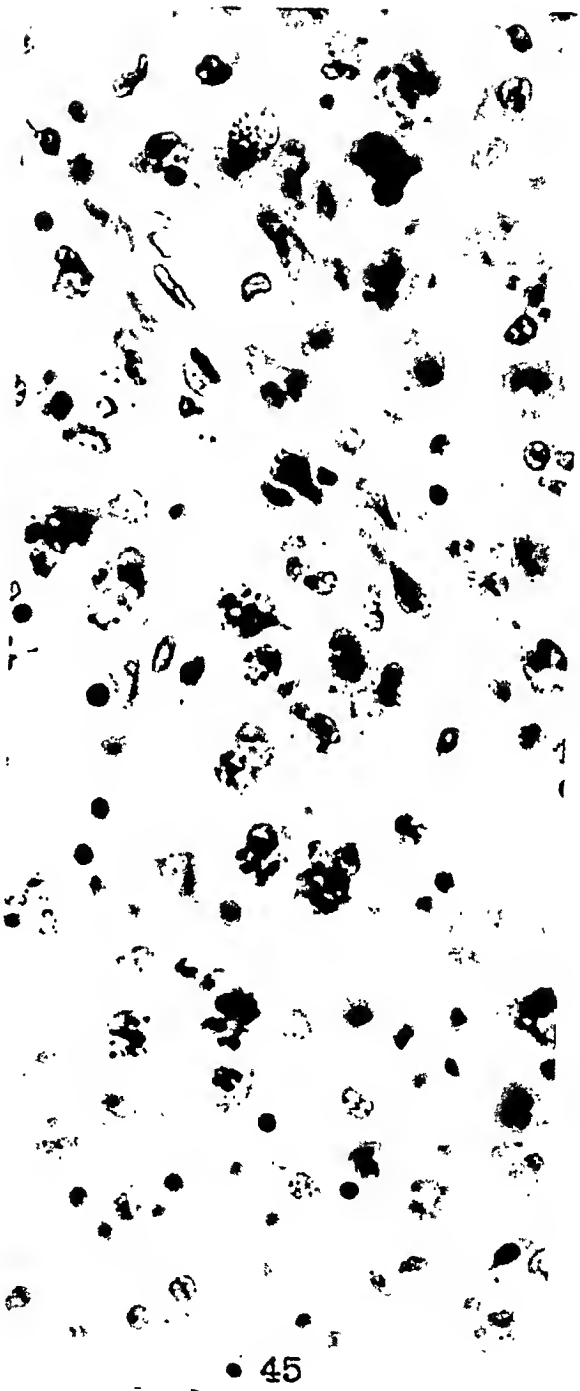
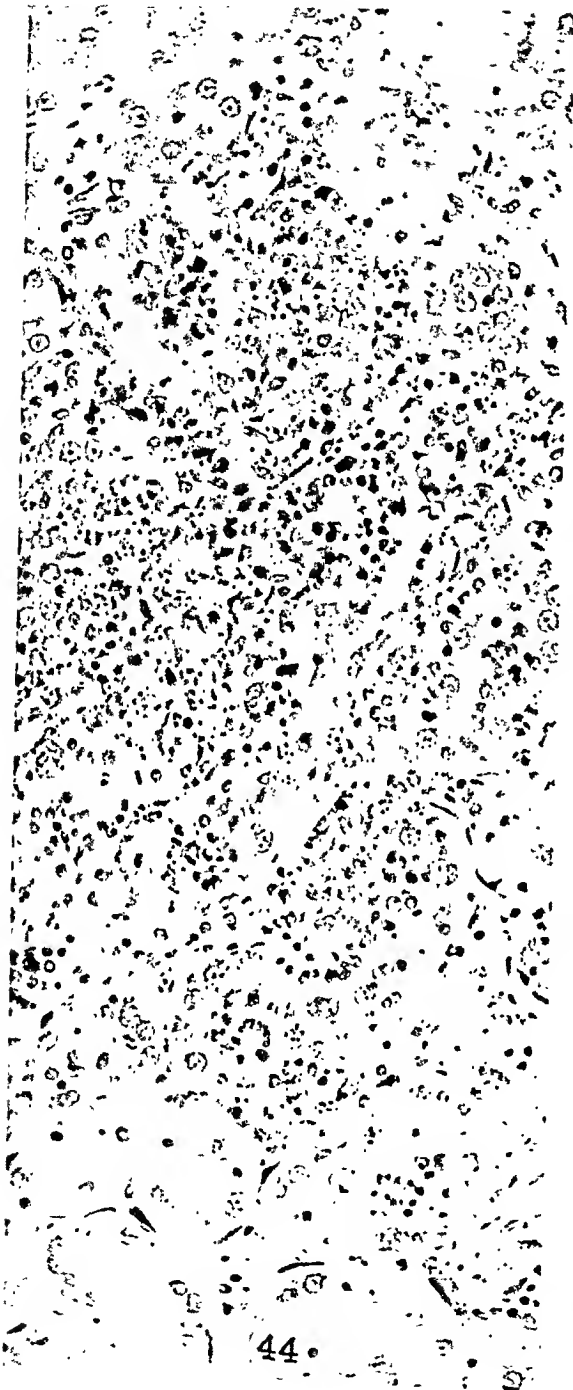


PLATE 228

- FIG. 46. Fatty products of degeneration, mostly in the perivascular space. Liver of a chick in the chronic stage of vitamin K deficiency. Sudan III stain.  $\times 140$ .
- FIG. 47. Perivascular edema and abundant fatty material from degeneration. Myocardium of a chick in the chronic stage of vitamin K deficiency. Sudan III stain.  $\times 100$ .
- FIG. 48. (a) Demyelination in a cerebellar folium of a chick in the chronic stage of vitamin K deficiency. Roizin's<sup>57n</sup> combined method for myelin sheath and lipid products of degeneration.  $\times 100$ .  
(b) Perivascular cerebral demyelination in a chick in the chronic stage of vitamin K deficiency. Roizin's<sup>57n</sup> combined method for myelin sheath and lipid products of degeneration.  $\times 80$ .

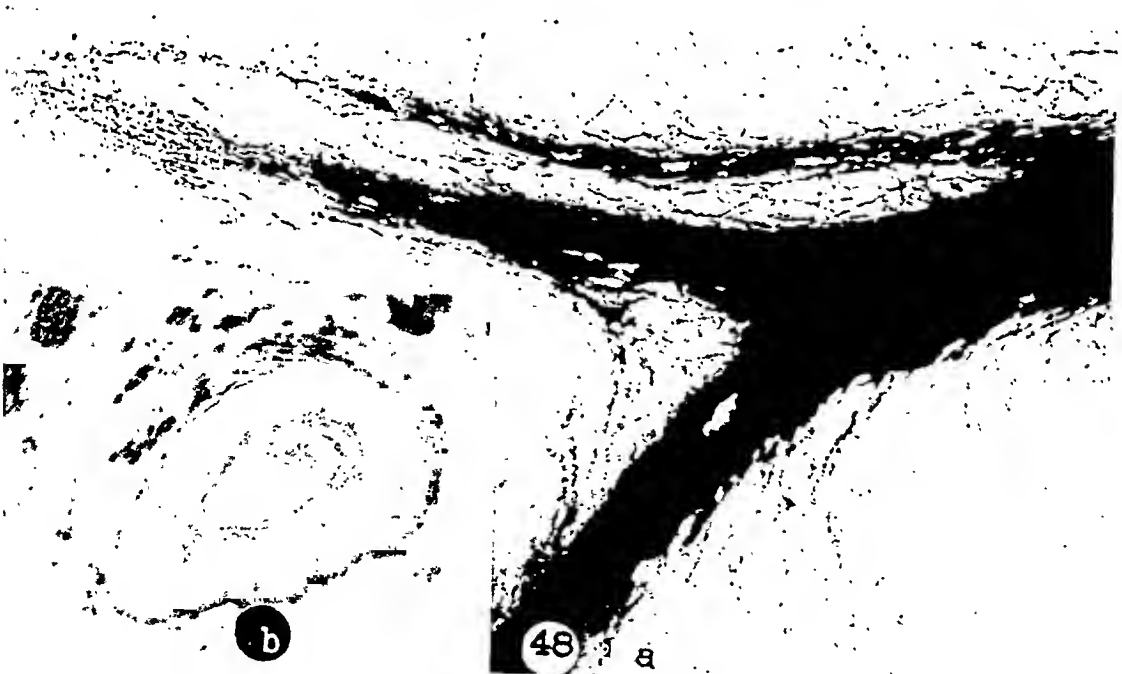


PLATE 229

- FIG. 49. Stratification of endothelial vascular cells. Cerebral blood vessel of a chick in the chronic stage of vitamin K deficiency. Nissl's stain.  $\times 190$ .
- FIG. 50. Hyperplasia and hypertrophy of the walls of a cerebral blood vessel of a chick in the chronic stage of vitamin K deficiency. Nissl's stain.  $\times 190$ .
- FIG. 51. Disintegration and almost complete disorganization of liver structure, and fibrosis. Chick in the chronic stage of vitamin K deficiency. Masson's trichrome stain.  $\times 360$ .

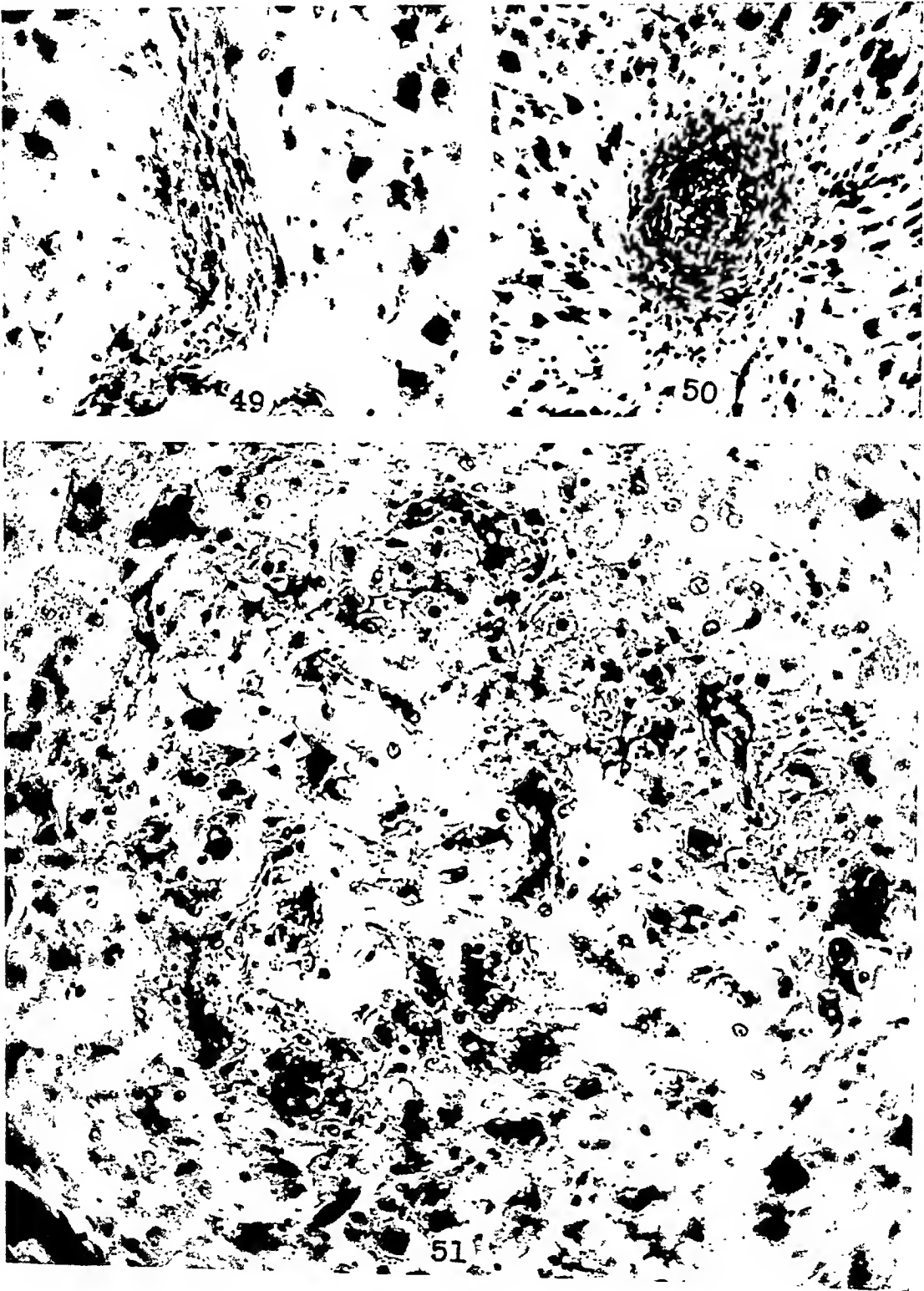




PLATE 230

- FIG. 52. Demyelination and debris of disintegrated myelin sheaths in long-standing cerebellar hemorrhagic area of a chick in the chronic stage of vitamin K deficiency. Roizin's <sup>57th</sup> combined method for myelin sheath and lipid products of degeneration.  $\times 190$ .
- FIG. 53. Areas of cellular destruction in the Purkinje and granular layers of the cerebellum in a chick in the chronic stage of vitamin K deficiency. Nissl's stain.  $\times 80$ .
- FIG. 54. Area of destruction with remains of hemorrhage, involving mostly the granular layer of the cerebellum. Chick in the chronic stage of vitamin K deficiency.  $\times 110$ .

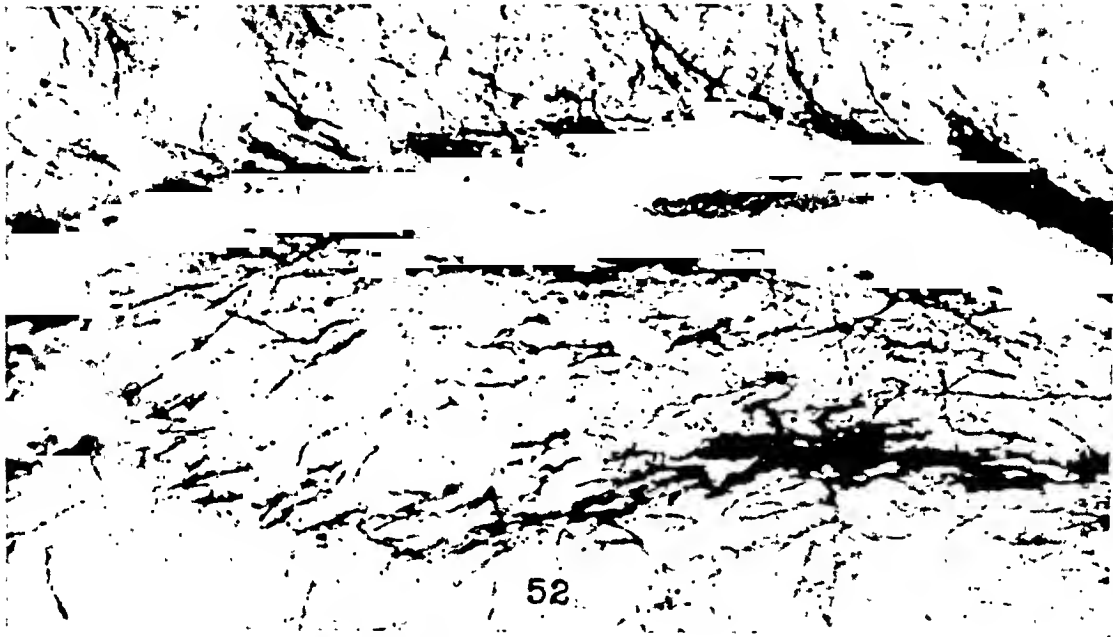


PLATE 231

FIG. 55. Area of reparative fibrosis in the liver of a chick in the chronic stage of vitamin K deficiency. Hematoxylin and eosin stain.  $\times 140$ .

FIG. 56. Small area of fibrosis with congested blood vessels in the liver of a chick sacrificed during the chronic stage of vitamin K deficiency. Masson's trichrome method.  $\times 100$ .

FIG. 57. High-power magnification of Figure 56, revealing details of the fibrosis. Hematoxylin and eosin stain.  $\times 360$ .

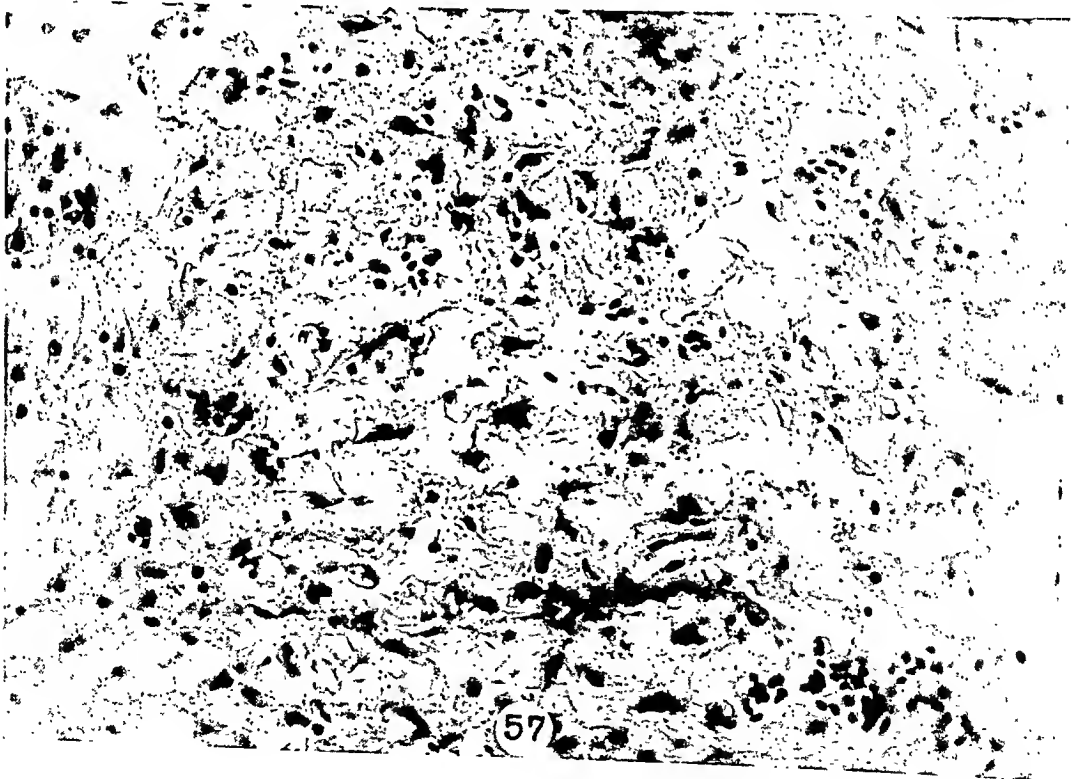
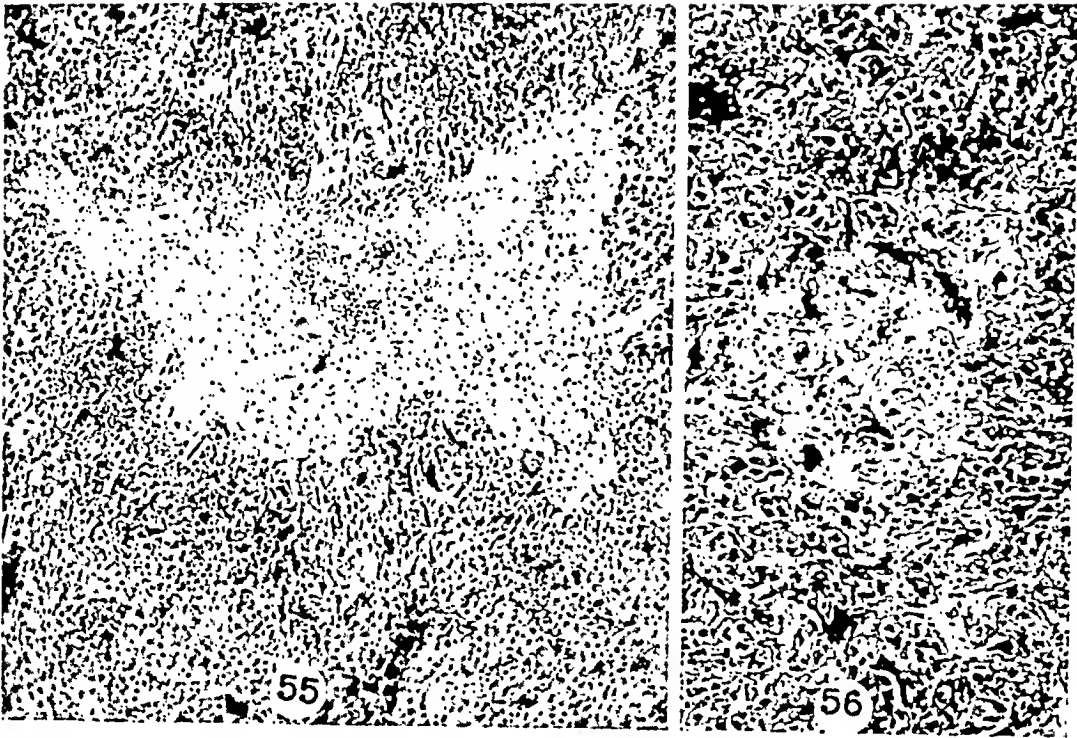


PLATE 232

- FIG. 58. Marked hypertrophy and hyperplasia of microglial cells in the central nervous tissue of a chick sacrificed during the chronic stage of vitamin K deficiency. Hortega's silver carbonate method.  $\times 360$ .
- FIG. 59. Hypertrophy and hyperplasia of astrocytes in an area of gliosis in a chick sacrificed in the chronic stage of vitamin K deficiency. Cajal's gold sublimate method.  $\times 220$ .
- FIG. 60. Organized subcutaneous hematoma of 14 months' duration in a rat sacrificed in the chronic stage of vitamin K deficiency.
- FIG. 61. Small, organized hematoma in the retrosellar region of a rat (second generation), 340 days old, resulting in clinical symptoms of Fröhlich's syndrome.
- FIG. 62. Marked depression at the base of the brain produced by the hematoma shown in Figure 61.



PLATE 233

FIG. 63. (a) Marked proliferation of cerebral capillaries in a chick which died in the subchronic stage of vitamin K deficiency. Nissl's stain.  $\times 152$ .

(b) Area of encephalomalacia in a chick sacrificed in the subchronic stage of vitamin K deficiency. Nissl's stain.  $\times 360$ .

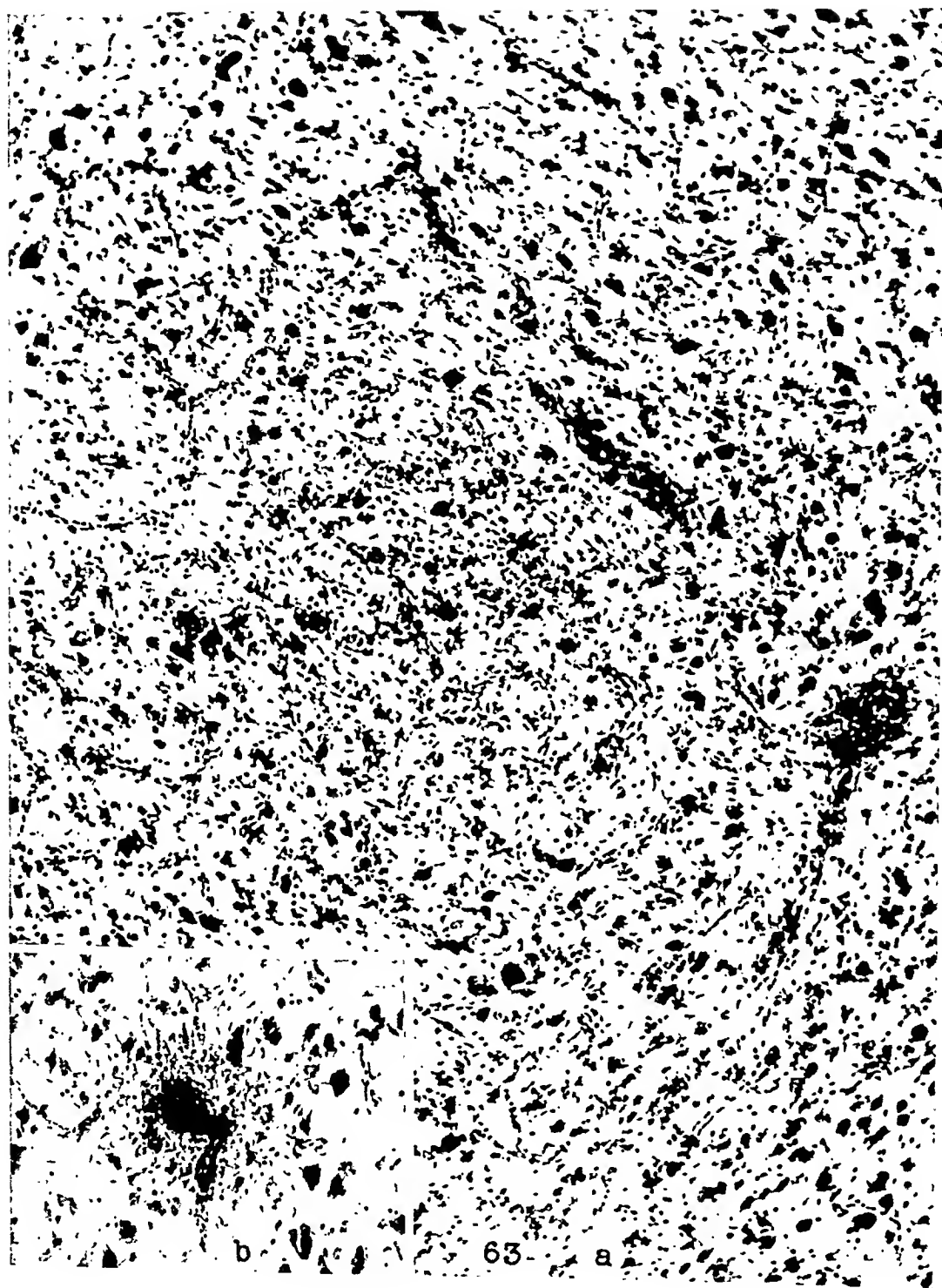




PLATE 234

FIG. 64. Deformity of the left knee joint and retraction of the leg in flexion in the subchronic stage of vitamin K deficiency.

FIG. 65. Marked swelling of the left knee joint in the subchronic stage of vitamin K deficiency.

FIG. 66. Involvement of both knee joints resulting in inability to walk and to stand in an erect position in the chronic stage of vitamin K deficiency.



PLATE 235

FIG. 67. (a) Lateral view of an anatomic preparation illustrating the slipping of the tendon from its normal position in a chick in the subchronic stage of vitamin K deficiency.

(b) Lateral view of an anatomic preparation illustrating the tendon of a control chick in its normal position.

FIG. 68. Posterior view of Figure 67a.

FIG. 69. Posterior view of Figure 67b.

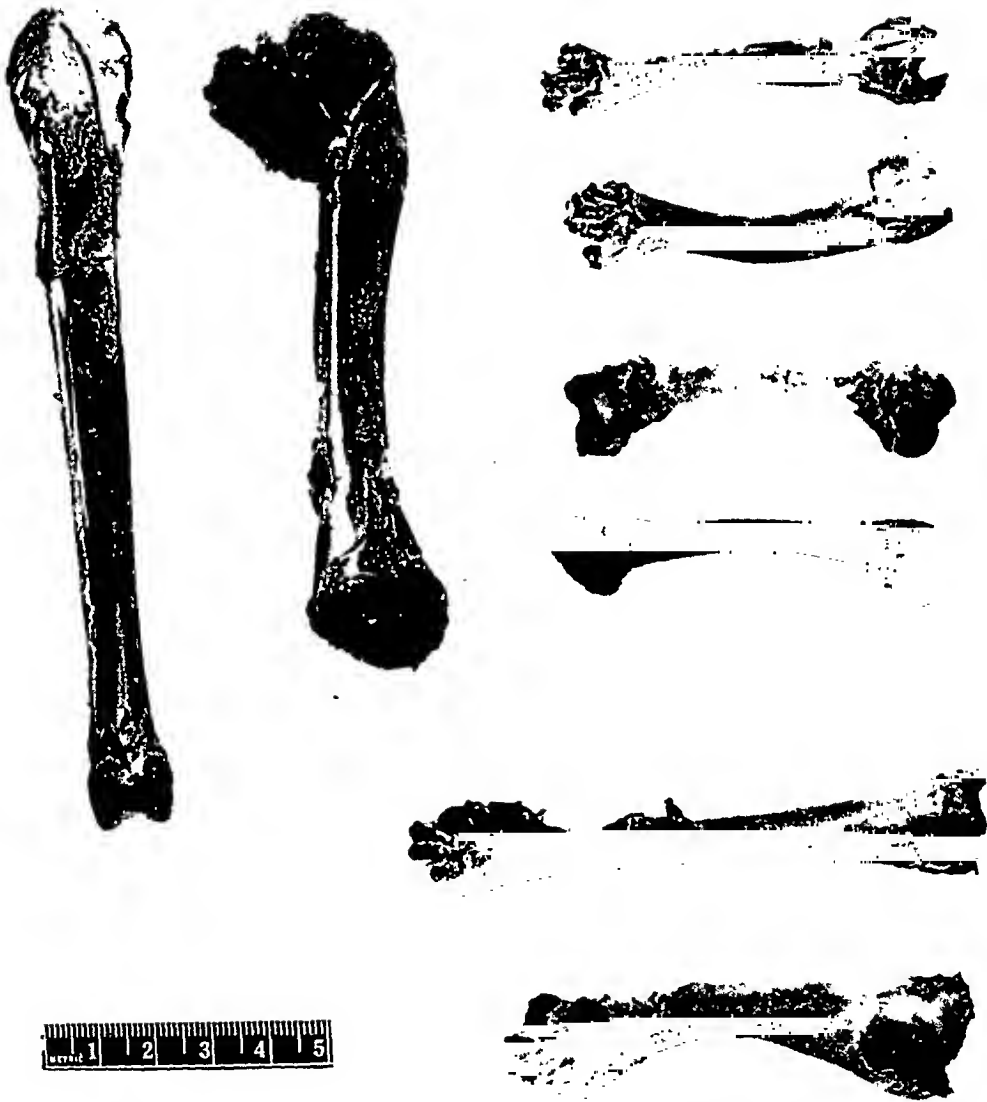


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Diathesis by Deficiency in Vitamin K

PLATE 236

FIG. 70. Various aspects of bone deformities and dystrophies in chicks in the subchronic and chronic stages of vitamin K deficiency.



70

PLATE 237

FIG. 71. Deformity and rupture of periarticular and intra-articular tissues of the knee joint of a chick in the chronic stage of vitamin K deficiency.  $\times 7$ .

FIG. 72. Deformity and dystrophy of epiphyseal cartilage of the knee joint of a chick in the chronic stage of vitamin K deficiency. Hematoxylin and eosin stain.  $\times 7$ .

FIG. 73. Hemorrhages in a knee joint (left side of figure) for comparison with a normal joint (right side of figure).

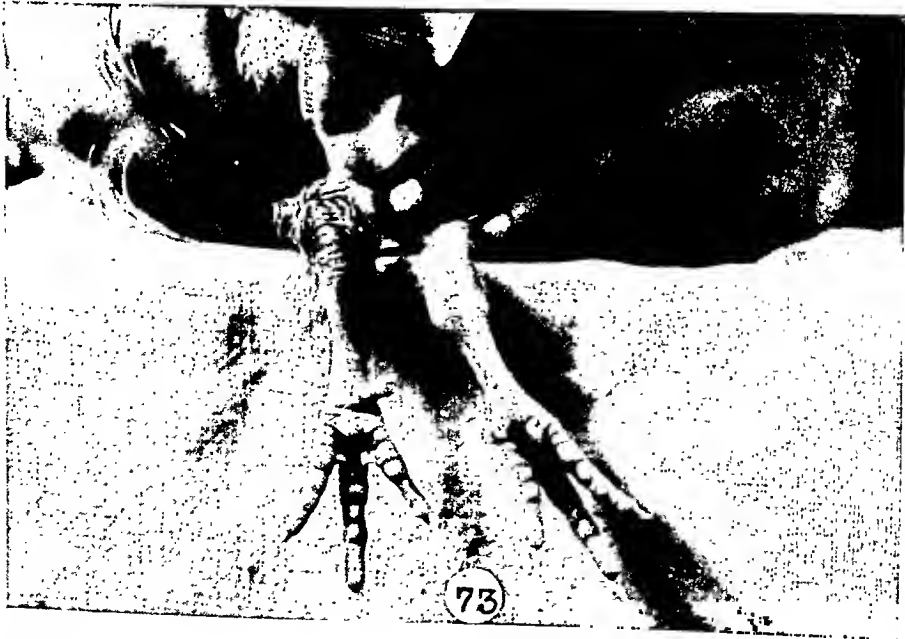
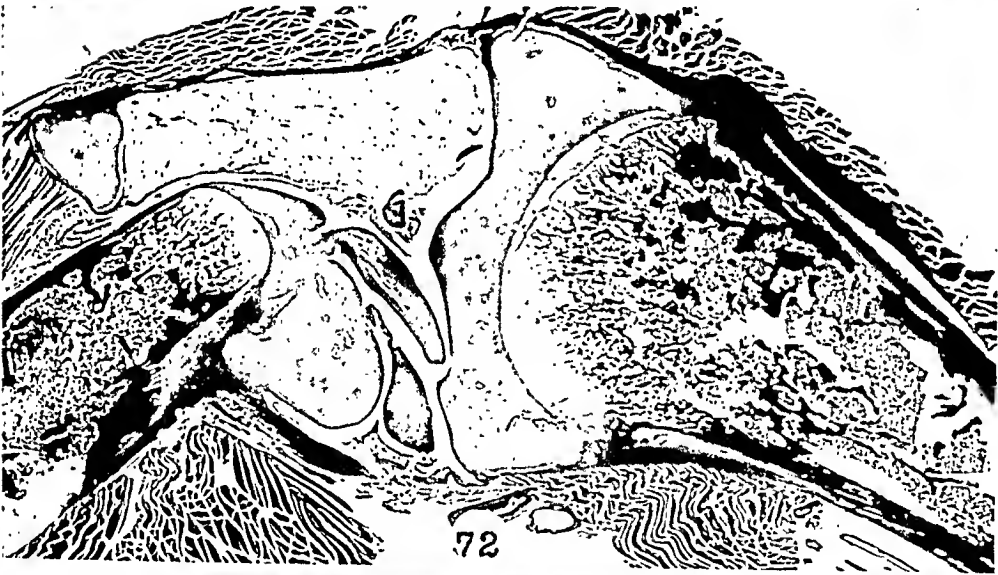
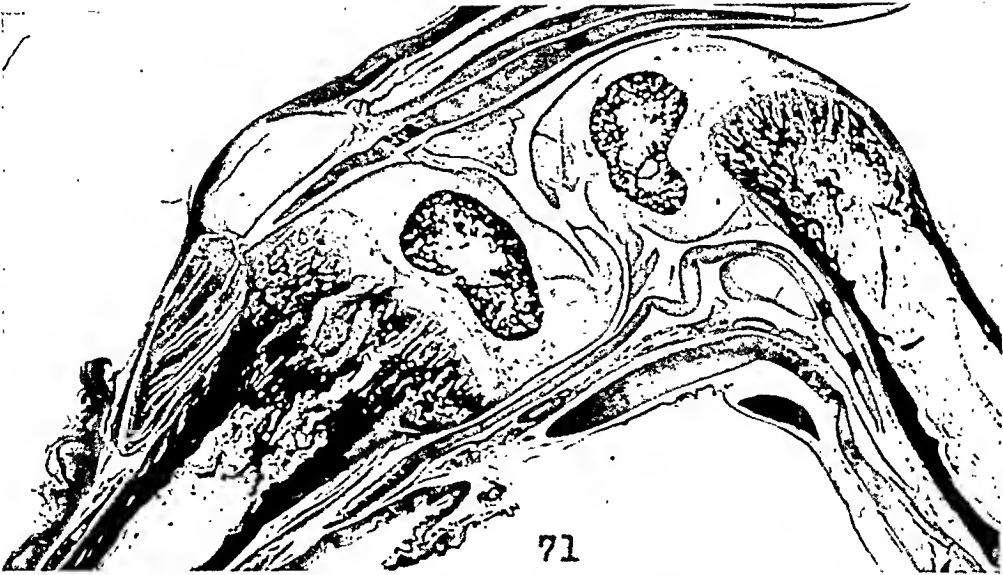
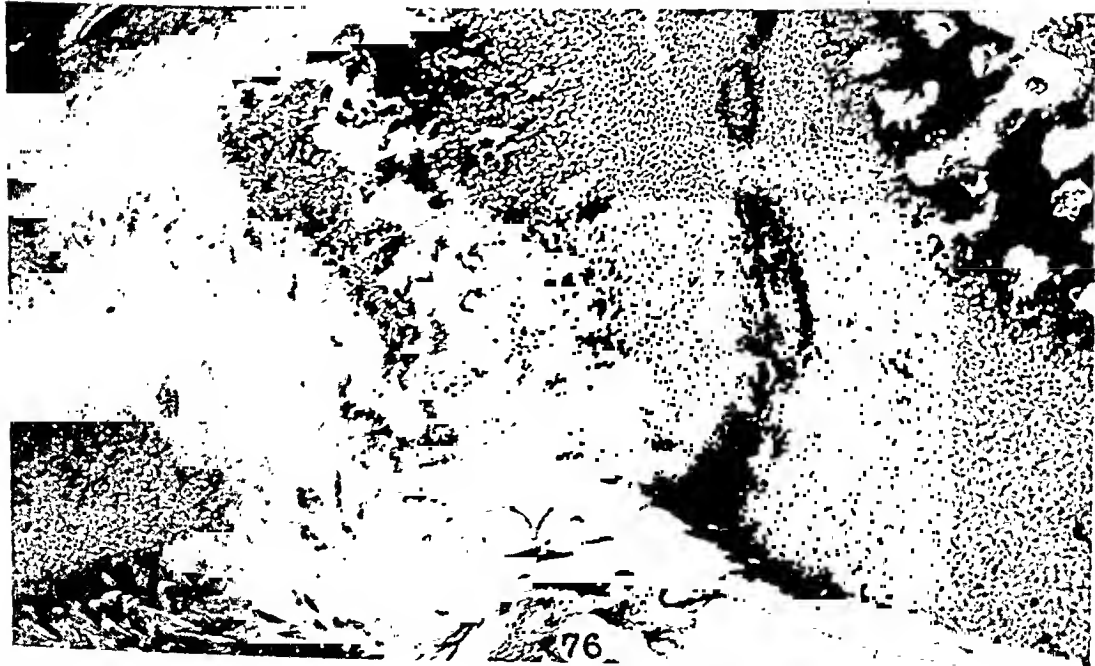
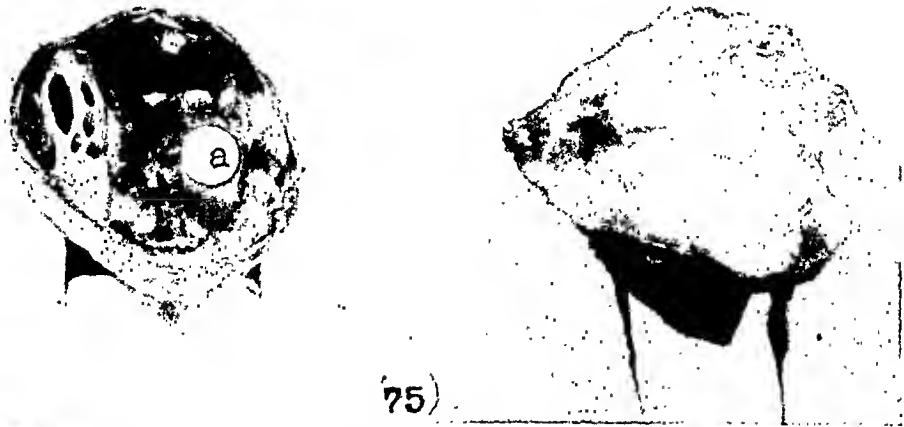




PLATE 238

- FIG. 74. External swelling and distention resulting from hemorrhage in the knee joint of a chick in the subchronic stage of vitamin K deficiency.
- FIG. 75. Anatomic preparation of the knee joint shown in Figure 74, revealing blood in the joint (a). The right side of the figure illustrates a normal control joint.
- FIG. 76. Large hemorrhage infiltrating the upper part of the diaphysis of a tibia resulting in the fracture of the bone plate up to the periosteum in a chick in the subchronic stage of vitamin K deficiency.





# STUDIES ON THE COAGULATION DEFECT IN A CASE OF THROMBOCYTOPENIC PURPURA COMPLICATED BY THROMBOSIS \*

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Recent investigations have demonstrated that thrombosis can be prevented by the administration of anticoagulant drugs.<sup>1</sup> It is therefore of interest to observe a case in which thrombosis occurred in the course of a hemorrhagic syndrome featured by marked prolongation of the coagulation time of the blood. We wish to report such a case, with experimental observations on the nature of the coagulation defect, and to give a brief review of the literature dealing with the coagulation time in thrombocytopenic purpura and the neurologic complications of that condition.

## THE COAGULATION TIME IN THROMBOCYTOPENIC PURPURA

Although the blood platelets yield the principal source of thromboplastin for the coagulation of shed blood, there is no significant correlation between the coagulation time and the platelet count. In a group of 139 paired determinations of these factors on the venous blood in experimental thrombocytopenic purpura in dogs, Tocantins<sup>2</sup> found an insignificant correlation of  $\text{minus } 0.234 \pm 0.069$ . In our group of 743 observations of 402 patients suffering from various diseases, the coefficient of association (Yule's Q) between the same two factors was  $0.20 \pm 0.16$ , showing no association in the group tested.<sup>3</sup>

In the great majority of cases reported in the literature, the coagulation time in thrombocytopenic purpura appears to have been within the normal range for the methods employed (usually under 10 minutes). However, Evans<sup>4</sup> found a slight prolongation of the coagulation time which returned to normal coincident with elevation of the platelet count in 8 patients who were splenectomized. Other cases in which the coagulation time was slightly or moderately prolonged (up to 25 minutes) have been observed.<sup>4,6,7,8</sup> Tidy<sup>5</sup> stated that "At present the fact is established that coagulation time is slightly, but not greatly, increased in haemorrhagic purpura. The reason for this is still uncertain."

In thrombocytopenic purpura a slight or moderate prolongation of the coagulation time is not unusual, but a *marked* prolongation has seldom been observed. Marzollo's<sup>9</sup> case was that of a 3-year-old girl. The platelets numbered between 10,000 and 80,000 per cmm., and the

\* Received for publication, December 15, 1945.

coagulation time, tested by various methods, was 23 to 160 minutes. As clinical improvement took place, the platelet count rose to 120,000 per cmm., and the coagulation time returned to normal.

The case reported by Aubertin and Lafon<sup>10</sup> was that of a man, 19 years of age. Examination 1 month before death showed a markedly prolonged bleeding time and a nonretractile blood clot. The platelets numbered 136,000 per cmm. and the coagulation time was 10 minutes. Five days before death the platelets numbered 182,000 per cmm. and the coagulation time was greater than 2 hours. On the day before death the coagulation time was markedly prolonged; after standing overnight at room temperature, coagulation had taken place only in that part of the specimen occupied by the sedimented erythrocytes; the supernatant plasma remained completely liquid.

#### NEUROLOGIC COMPLICATIONS OF THROMBOCYTOPENIC PURPURA

Intracerebral hemorrhage has been the most frequently reported neurologic complication of thrombocytopenic purpura.<sup>11-21</sup> In some patients the hemorrhages have been large and solitary; in some, small and multiple; and in others they have ruptured into the subdural space or into the ventricles. In addition there have been many reports of primary subdural or subarachnoid hemorrhage.<sup>10,18-24</sup> Unlike hemophilia, a number of cases of cerebellar hemorrhage have been encountered in thrombocytopenic purpura.<sup>18,20</sup> Less commonly, hemorrhages have occurred in the midbrain,<sup>18,25</sup> the pituitary gland,<sup>26</sup> the optic tracts,<sup>25,27</sup> the spinal cord,<sup>28,29</sup> and the spinal meninges.<sup>18</sup> No reports of peripheral nerve involvement in thrombocytopenic purpura have come to our attention. This is in contrast to hemophilia, in which such lesions are rather frequently encountered.<sup>30</sup> We have found no reports indicating a disturbance in the cranial venous sinuses in thrombocytopenic purpura except in the case reported by Welt and Kasnetz<sup>33</sup> in which thrombocytopenic purpura occurred as a complication of acute mastoiditis with lateral sinus thrombosis, *Streptococcus viridans* bacteremia, and multiple metastatic abscesses.

#### REPORT OF CASE \*

W. J. F. (no. U 79071) was a white male machine operator, 29 years of age, who entered the University of California Hospital on December 4, 1941.

*History.* The family history was irrelevant for bleeding diseases. No cause of secondary thrombocytopenic purpura was discovered in the past history. At the age of 19 years the patient had suffered from toxic hepatitis following the administration of neoarsphenamine given for an indolent ulcer of the left lower leg. The ulcer persisted, and in 1939 and 1940 additional ulcerated areas appeared on the dorsal surfaces of both feet. In recent months the lesions had healed, leaving

\* A brief summary of this case has been published previously.<sup>49</sup>

heavily pigmented scars. He had had a nonproductive cough for many years and for the past 3 years had suffered from dyspnea on exertion, ankle edema, and nocturia. For the past 6 months he had experienced increasing "stiffness" of the leg muscles. The patient had bruised easily since childhood. During the past year bouts of epistaxis had occurred with increasing frequency and severity and numerous petechial hemorrhages had appeared in successive showers over the lower extremities. He had also noted bright red blood in the stool on numerous occasions.

*Physical Examination.* The patient was undernourished, fairly well built, pallid, and appeared older than his stated age of 29 years. There was a generalized purpuric eruption, most marked over the lower extremities and characterized by innumerable petechiae and many purpuric maculae as large as 1 cm. in diameter. There were large scarred areas over the lower part of the left foreleg and ankle and over the dorsa of both feet, where the skin was thin, scaly, deeply pigmented, and fixed to the underlying tissues. The lymph nodes were not enlarged. In the right optic fundus there was a large, fresh hemorrhage and several areas of absorbing exudate. The nasal passages were obstructed with crusted blood and there was continuous oozing from the right nostril. The mouth was edentulous and there were numerous small hemorrhages in the oral and pharyngeal mucous membrane. The lungs and heart were within normal limits. The pulse rate was 100 per minute and the blood pressure was 150/95 mm. Hg. The peripheral arteries were soft and pulsated normally. The spleen descended two fingersbreadth below the left costal margin, but no other abdominal organs or masses were palpable. The right testicle was in the inguinal canal. Prolapsing internal hemorrhoids were found on rectal examination. The fingers were slightly clubbed and there was slight thoracic kyphosis, but the remainder of the skeletal system was normal. Examination of the nervous system revealed no abnormalities.

*Laboratory Findings.* The hematologic findings are given in Table I, and normal values for the hemostatic tests employed are given in Table II. There was a marked anemia, probably due to continuous blood loss. The leukocyte count varied between 2,900 and 6,400 per cmm. The differential count was normal except for a slight increase in the percentage of polymorphonuclear neutrophils. The platelet count varied between 80,000 and 250,000 per cmm. Furthermore, it was noted that the platelets were large, stained poorly, and did not contain granules. The bleeding time was markedly prolonged, the clot retraction very poor, and the capillary fragility definitely increased. The prothrombin concentration was slightly below normal on the patient's entry to the hospital and fell to 40 per cent of normal during the ensuing 3 weeks. It rapidly returned to normal following the administration of vitamin K. The coagulation time of the venous blood, tested at room temperature, was moderately prolonged when the patient was first observed and subsequently became markedly prolonged. The coagulation time of the venous blood at 37° C. was tested only during the last week of life when it was found to be moderately prolonged.

It should be pointed out that, while there is only a slight difference between the normal values for the coagulation time at room temperature and at 37° C., there is a marked difference between their pathologic ranges. In parallel tests done on the same blood specimens it has been found that coagulation time of 5 to 10 hours at room temperature will be reduced to 1 to 2 hours at 37° C.<sup>44</sup> The experimental observations relating to the coagulation defect were made on January 5 and 7, 1942, and are discussed below.

The urine was grossly bloody, specific gravity was 1.023, albumin was positive (3 plus). The stool gave a positive test for occult blood (4 plus). The plasma

TABLE I  
*Results of Hematologic Tests*

Date	Hemoglobin in gm. per 100 cc.	Erythrocytes in millions per cmm.	Leukocytes per cmm.	Differential white blood cell count in per cent					Platelets per cmm.	Bleeding time in minutes
				Polymorphonuclear cells		Polymor- phonuclear eosinophils	Lymph- ocytes	Mono- cytes		
				Fil.	Non- Fil.					
12-4-41	7.7	2.68	5300	60	4		20	16	80,000	30
12-5-41									70,000	
12-10-41									90,000	
12-14-41									100,000	
12-16-41	6.1	1.88	4300	74	8	1	10	7	180,000	
12-20-41			4600						170,000	
12-21-41									190,000	
12-23-41										
12-24-41	6.9	2.53	4300						160,000	10½
12-26-41	6.9	2.50	3900						170,000	
12-27-41	7.4	2.58	3200						120,000	
12-29-41	7.7	2.45	4400						130,000	
12-30-41	7.7	2.60	3100						230,000	30
12-31-41	8.4	2.74	3400						200,000	
1-2-42	8.4	3.01	3900						250,000	30
1-3-42	7.6	2.78	2900						200,000	
1-5-42	7.7	3.01	3700						160,000	30
1-6-42	7.7	3.22	4500						180,000	
1-7-42									100,000	
1-8-42	7.2	2.70	4900	46	35		11	8	140,000	

TABLE I—continued

Date	Coagulation time of venous blood at room temperature in minutes	Coagulation time of venous blood at 37° C. in minutes	Fluid volume of clot in per cent	Prothrombin concentration in per cent of normal	Capillary fragility in numbers of petechiae						Citrated whole blood transfusions in cc.		
					Arm		Thigh						
					mm. Hg suction		mm. Hg suction						
					150		100		150			200	
					150	200	150	100	150	200			
12-4-41	24		50	75	15	Shower*	Shower	Shower	Shower	300			
12-5-41									500				
12-10-41									500				
12-14-41									500				
12-16-41	42		44	40	2	2	1	Shower	500				
12-20-41									500				
12-21-41													
12-23-41													
12-24-41	105		43	45†	1	4	6	Shower	500				
12-26-41													
12-27-41													
12-29-41													
12-30-41	175		43	70	0	0	Shower	Shower	500				
12-31-41													
1-2-42													
1-3-42													
1-5-42	130	23½	44	80	9	10	Shower	Shower	500				
1-6-42													
1-7-42													
1-8-42													

\* "Shower" indicates that the petechiae were too numerous to count accurately; usually from 50 to several hundred were present.

† Two mg. Synkamin (4 amino-2 methyl-1 naphthol) Parke, Davis &amp; Co., administered intravenously on 12-30-41 and daily thereafter.



fibrinogen was 1.17 gm. per 100 cc. The serum calcium was 9.6 mg., serum phosphorus, 5.43 mg., and nonprotein-nitrogen of the blood, 32.9 mg. per 100 cc. The Wassermann and Kahn reactions of the blood were negative. The icterus index was 9 units and the intravenous hippuric acid test for liver function gave a normal value (1.09 gm.). The intravenous phenolsulfonplthalein test of kidney function showed 70 per cent excretion of the dye in 2 hours. The tuberculin (1:10,000) skin test was negative. The patient belonged to blood group A.

*Course of Illness.* The patient received 4,300 cc. of citrated blood by transfusion (Table I). Numerous thrombi developed in the vessels used for venipuncture. Between December 9 and December 29, 1941, the patient received roentgen irradiation of the spleen (a total of 1200 r. to each of two areas). Epistaxis continued to recur despite repeated nasal packing and cauterization of bleeding points in Kiesselbach's area. The urine and stool were grossly bloody throughout most of his hospital stay. On December 8th the right malar region became edematous and markedly tender. Roentgenograms showed complete opacity of the right antrum and ethmoid cells without evidence of bone destruction. The condition persisted for about a week and was interpreted as due to hemorrhage into the antrum. On December 19th the patient complained of sore throat and difficulty in swallowing, and he was found to have a large submucous hemorrhage in the posterior wall of the pharynx, extending down into the tissues of the neck. While this hemorrhage was resorbing, he began to have a daily temperature elevation, reaching a maximum of 41°C. (rectal) on December 22nd. Although no abnormal physical signs were detected in the chest, roentgenograms showed patchy areas of increased density throughout most of the right lung field. During the following 8 days a total of 42 gm. of sulfadiazine were administered orally and the temperature gradually returned to normal. On January 6, 1942, the patient complained of pain

TABLE II  
*Normal Values for Hemostatic Tests\**

Test	Mean	Standard deviation	Normal range
Bleeding time (Ivy), in minutes	3.2	1.6	0 to 6.4
Coagulation time of venous blood at room temperature in minutes	8.9	2.7	3.5 to 14.3
Coagulation time of venous blood at 37°C. in minutes	7.5	1.4	4.7 to 10.3
Clot retraction. Fluid volume per cent of clot†	7.9	6.0	4.1 to 19.9
Platelet count per cmm.	409,000	68,000	273,000 to 545,000
Prothrombin concentration (Quick) in per cent of normal			75 to 100
Capillary fragility (Dalldorf). Number of petechiae appearing on arm with 200 mm. Hg vacuum pressure			0 to 10

\* The coagulation time at 37°C. was determined on a group of 40 normal subjects. All other tests were performed on a group of 64 normal subjects. (This is the same group used by us in previous analyses<sup>40,50</sup> except that in a more critical clinical analysis 36 subjects, formerly considered to be normal, were excluded because their normality was questioned. This resulted in no statistically significant differences in the values for the mean, standard deviation, or normal range.) The standard statistical method was used in setting up the limits of normality for the bleeding time, coagulation time, fluid volume of the clot, and platelet count. The limits of significance of the data were set at two standard deviations from the mean, thereby including approximately 96% of the observations. The mean is taken at the point of reference. All measures which are calculated were at least three times their sampling errors. The limits of normality for the prothrombin concentration and capillary fragility were arbitrarily set by direct observation. The prothrombin concentration in 91% of the observations fell between 75% and 100%. In the capillary fragility test not more than 10 petechiae appeared in 92% of the observations. The techniques for the tests here employed are described in references 34, 43, 48, and 49.

† Formerly called the "Extracorporeal volume per cent" of the clot.

in the right upper abdominal quadrant, which by the following day had extended into the right lower chest and was referred to the right shoulder. On the evening of January 10th the patient complained of marked headache over the vertex. His neck was stiff and painful on attempted flexion, but he had no other abnormal neurologic signs. By the following morning he was in coma and had moderate bilateral symmetric proptosis, and marked boggy edema of the entire scalp, nuchal region, forehead, eyelids, and tongue. At 2:00 P.M. lumbar puncture was performed. The initial pressure was greater than 1,000 cm. of water, and after removal of 70 cc. of grossly bloody fluid the pressure was 750 cm. of water. There was no immediate change in the patient's condition and he expired 1 hour later.

#### AUTOPSY FINDINGS

Autopsy was performed 2 hours after death. Post-mortem lividity had appeared, but rigor mortis was absent. The entire scalp was thickened, boggy, and edematous, and the coronal portion was infiltrated with blood. There was marked edema of the eyelids, the under side of the tongue, and the posterior portion of the neck. There was moderate symmetric proptosis of the eyes, which were deviated upwards and divergent.

There was a small area of hemorrhage in the fat of the anterior mediastinum. The right pleural cavity contained 750 cc. of yellow, turbid fluid. There were numerous subpleural hemorrhages, bilaterally. The largest (8 cm. in diameter) was beneath the right posterior basal parietal pleura and the pleural membrane adjacent to it was thickened. The right lung weighed 500 gm.; the left lung, 300 gm. The major bronchi and pulmonary vessels were normal. Both lungs were congested and edematous, but there was no gross hemorrhage or consolidation.

The peritoneal cavity was normal. A portion of the omentum was adherent to the gallbladder. The liver weighed 2,200 gm. The anterior edge was rounded and the cut surface pale. The gallbladder and bile ducts were normal. The spleen weighed 340 gm.; its capsule was reddish gray and slightly wrinkled; numerous large, white lymphoid follicles were visible on the cut surface. The splenic pulp was pale red and could be scraped readily from the cut surface. The pancreas was normal. The entire gastrointestinal tract was normal except for a few tiny, mucosal hemorrhages in the stomach. The kidneys each weighed 260 gm. The corticomedullary differentiation was indistinct. Numerous small (1 to 2 mm.) hemorrhages were present throughout the parenchyma and beneath the capsule. The renal pelves and calyces, ureters, bladder, and prostate gland were normal. The undescended right testicle was half the normal size.

The heart weighed 340 gm. and was normal except for hemorrhagic infiltration of the right auricular wall and numerous atheromatous

plaques in the coronary arteries, one of which, situated 1 cm. from the mouth of the left coronary artery, partially occluded its lumen. There were a few small, atheromatous plaques in the abdominal aorta.

The pituitary body, thyroid gland, and adrenal glands were normal. There was an irregular, small (0.5 cm.) polypoid mass, containing several small mucoid cysts, projecting from the left vocal cord. The mediastinal and aortic lymph nodes were moderately enlarged, soft and congested. The marrow of the ribs and lumbar spine and sternum appeared grossly normal.

The brain weighed 1,310 gm. The cerebral convolutions were flattened and the cerebellar tonsils were prominent. There was an extensive subarachnoid hemorrhage around the base of the brain with a small amount of blood over the cortex. No other abnormalities were noted in the brain, but both cavernous sinuses were completely filled with firm, adherent, thrombotic material. The venous sinuses at the base of the skull were partly occluded by similar thrombi.

### *Microscopic Examination*

Only those tissues which showed histopathologic changes will be described in detail. The heart, liver, gallbladder, pancreas, gastroenteric tract, and thyroid, parathyroid, prostate, and thymus glands were normal.

The pulmonary alveoli contained large numbers of pigment-filled macrophages. In some there were masses of condensed, hyalinized fibrin with early organization and many were atelectatic. A section of the right posterior basal parietal pleura showed that the pleura, subpleural connective tissue, and adjacent muscle were edematous and infiltrated with red blood cells and masses of hyaline fibrin with early organization.

The epithelium of the larynx was elevated. In the submucosa there was extensive infiltration with fresh red blood corpuscles and masses of hyaline fibrin and one zone of vascular granulation tissue containing pigment-filled macrophages. A majority of the numerous large veins contained organizing thrombi.

The malpighian bodies of the spleen were not enlarged nor well outlined. In the walls and in the lumina of the sinuses, reticulo-endothelial cells and macrophages were unusually prominent, and in the follicles these cells had replaced most of the lymphocytes. Both the sinuses and intersinusoidal spaces contained a moderate amount of blood, many polymorphonuclear leukocytes, a few neutrophilic myelocytes and plasma cells, a rare megakaryocyte, but no nucleated red blood corpuscles. No phagocytosis was demonstrable.

In the kidneys the majority of the glomeruli were normal; some were partially or completely hyalinized; some presented pericapsular fibrin deposits; and a few showed epithelial or fibrous capsular crescents. The afferent arterioles of occasional glomeruli were filled with hyaline thrombi. There were numerous, small, interstitial hemorrhages both recent and old, and many tubules contained blood or serous fluid. Collections of interstitial lymphocytes and small calcium deposits were noted.

There were a few small hemorrhages in the submucosa of the bladder which was infiltrated with many large lymphocytes and macrophages and a smaller number of neutrophilic leukocytes and plasma cells. There was considerable tubular atrophy of the right testis; however, interstitial cells were present in normal numbers. The urethral submucosa was moderately infiltrated with lymphocytes, large macrophages, and neutrophilic leukocytes.

The pituitary gland showed moderate hemorrhage into the capsule and the posterior lobe.

There was a small, recent, cortical hemorrhage in one adrenal gland.

Epidermis from the ankle was normal. The dermis was composed of dense, hyalinized, acellular fibrous tissue containing scattered lymphocytes and pigment-filled macrophages.

Mediastinal, peri-aortic, and other lymph nodes had essentially the same histologic structure. The peripheral follicles were large and fairly distinct. Except for a rim of lymphocytes, they consisted of large numbers of reticulo-endothelial cells and macrophages, a few plasma cells, and many nuclear fragments. The sinuses were prominent and dilated, and there was proliferation of the lining reticulo-endothelial cells. In the sinusoids there were numerous large macrophages, many of which contained blood pigment, and fewer lymphocytes, mast cells, plasma cells, and eosinophilic and neutrophilic leukocytes.

The relative quantity of fat cells, and of erythropoietic and myelopoietic tissue in the bone marrow was normal (Fig. 1). Approximately 20 per cent of the megakaryocytes had large, well formed, vesicular, multilobar nuclei and abundant, finely granular, eosinophilic cytoplasm without pseudopods. There was moderate shrinkage of the cytoplasm and nucleus of about 40 per cent of the megakaryocytes, and the remaining 40 per cent consisted of irregular, shrunken, basophilic nuclear masses surrounded by extremely scanty cytoplasm. An average of one megakaryocyte was present per high-power field.

No histologic alteration of the central nervous system was noted except for the presence of red blood cells and blood-pigment-containing macrophages in the subarachnoid space.

The fibrous septa, nerves, and ganglia of the cavernous sinuses were infiltrated with blood which had become condensed and amorphous. A surrounding chronic inflammatory reaction had occurred. The venous channels were narrowed and almost all of them were filled with thrombi (Figs. 2 and 3). The older thrombotic masses were composed of hyalinized fibrin and red blood corpuscles. Many of these thrombi had undergone early organization. The most recently formed thrombi consisted of platelet masses containing leukocytes and erythrocytes (Fig. 4). There was no apparent primary alteration of the endothelium.

An atherosclerotic process with calcification had greatly narrowed the lumina of both branches of the left coronary artery. There was minimal atherosclerosis of the aorta. There was marked alteration of small arteries (50 to 200  $\mu$ ) in the myocardium, lungs, kidneys, gallbladder, urinary bladder, spleen, and in the capsules of the pituitary and adrenal glands. Some of these vessels showed hyperplasia of the media while others presented a fibrous intimal thickening. An occasional vessel of this size had a hyalinized wall, but none contained thrombi. The arterioles were not altered.

### *Pathologic Diagnoses*

(1) Primary thrombocytopenic purpura with hemorrhage in the subarachnoid space, in the fibrous septa and nerves and ganglia of the cavernous sinuses, and in the mediastinum, lungs, subpleural space, right auricle, kidneys, stomach, adrenal gland, vocal cord, scalp, and skin. (2) Thrombosis of the basilar and cavernous sinuses, and of veins in the arms and laryngeal submucosa. (3) Generalized arteriosclerosis with atherosclerosis of coronary arteries and aorta, and hyperplastic arteriosclerosis involving small arteries of the myocardium, lungs, gallbladder, and the capsules of the pituitary and adrenal glands.

### DISCUSSION

#### *The Hemostatic Defect*

The clinical manifestations of hemorrhagic diathesis in this patient were those commonly found in purpura haemorrhagica and the results of the hemostatic tests were characteristic of that condition; *i.e.*, a low platelet count associated with markedly prolonged bleeding time, diminished blood clot retraction, and increased capillary fragility. In addition, the coagulation time of the blood was markedly prolonged. The most probable cause of the coagulation defect was hypothromboplastinemia. However, detailed investigations were performed in order

to exclude all other known causes of delayed coagulation of the blood.

Hypocalcemia was readily eliminated as a diagnostic possibility by the finding of a normal concentration of the serum calcium and by the inability of added calcium chloride solution to shorten the coagulation time.

A defect in coagulation due to deficiency of fibrinogen was likewise excluded by the finding of a high plasma fibrinogen concentration and by the appearance of ample quantities of formed fibrin in whole blood or plasma clots.

The initial prothrombin concentration was at the lower limit of normal and dropped to 40 per cent of normal during the ensuing 3 weeks. This possibly was due to continued blood loss and to specific dietary deficiency. It rapidly returned to normal following the administration of synthetic vitamin K. The fluctuations in the prothrom-

TABLE III

*Coagulation Times of the Blood of W. J. F. Following the Addition of Protamine*

Added protamine solution	Coagulation time of 2 cc. blood specimens of W. J. F.'s blood at 37°C.
(mg.%)	(minutes)
0.00	21
0.001	22
0.005	27
0.010	31.5
0.050	52.5
0.100	120

\*Each concentration made up in 0.1 cc. of 0.85% NaCl solutions.

bin concentration appeared to bear no relationship to the prolonged coagulation time and, furthermore, the prothrombin concentration was at no time low enough to account for the delayed coagulation of the blood.<sup>34-39</sup>

Coagulation defects due to the circulation of anticoagulant substances in the blood are rare. A moderately prolonged coagulation time, presumably due to retention of toxic substances by the diseased kidneys, is sometimes found in severe uremia. In this patient there was no evidence of renal failure and the concentration of nonprotein nitrogen of the blood was normal.

The coagulation time can be markedly prolonged by the intravenous administration of heparin, and the anticoagulant effect of heparin can be neutralized by the addition of protamine to the blood.<sup>40,41</sup> The possibility that the coagulation defect in this patient might be due to an excess of heparin in the blood was eliminated by the addition of protamine to his blood *in vitro* (Table III). The prolonged coagulation time was not shortened by this procedure.

A prolonged coagulation time due to an unidentified circulating anti-coagulant was recently reported by Lozner, Jolliffe and Taylor.<sup>42</sup> The plasma of their patient produced a marked prolongation of the coagulation time of normal blood when added in ratios of 1:10 and 1:20. In our patient no significant anticoagulant effect could be demonstrated by similar tests (Table IV). However, these experiments do not completely exclude the possibility of the presence of a circulating anti-coagulant. If such an anticoagulant were present in a relatively weak concentration, its effect might remain undetected by the technic employed.

TABLE IV  
*Coagulation Times of the Blood of a Normal Subject Following the Addition of Graded Quantities of the Plasma of W. J. F.*

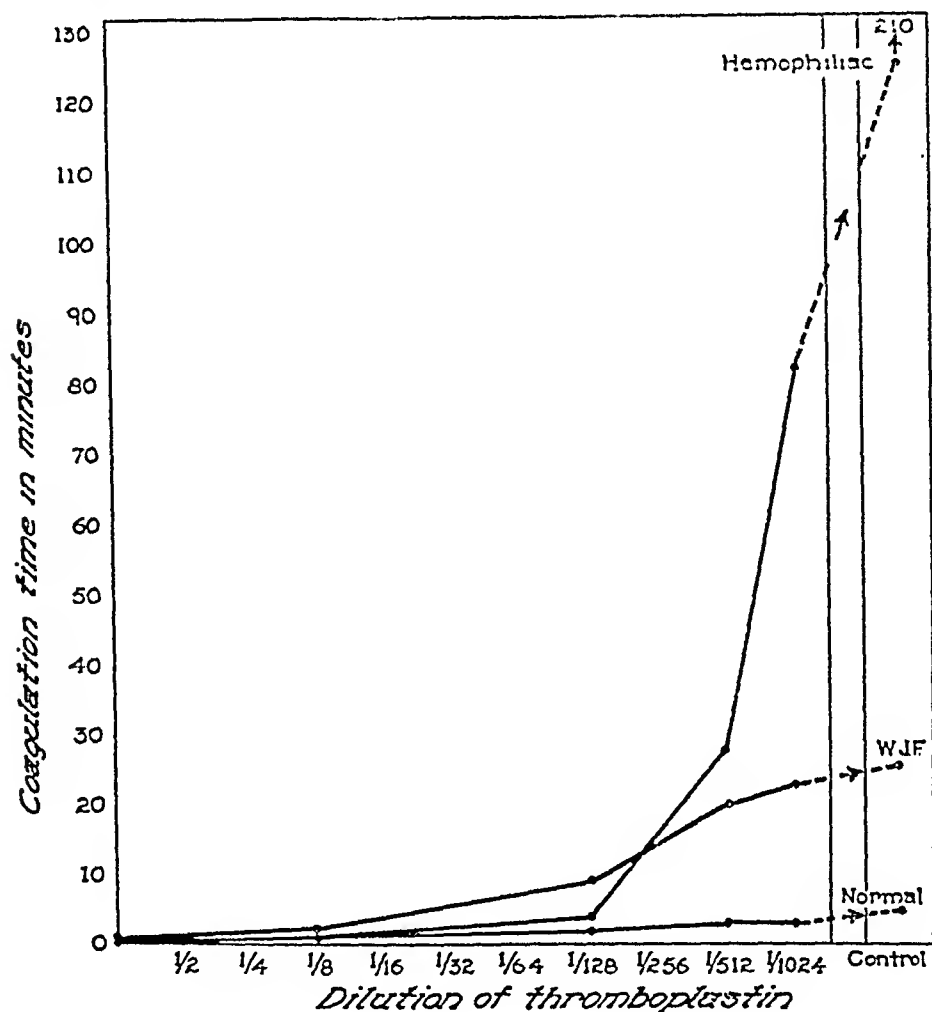
Added citrated plasma of W. J. F.	Coagulation time of 2 cc. specimens of normal blood at 37°C.
(cc.)	(minutes)
0	8.5
0.02	10
0.05	9
0.10	11.5
0.20	11

The possibility of the presence of both thrombocytopenic purpura and hereditary hemophilia in this patient seemed remote since his clinical history was not that of hemophilia and his family history gave no support to such a diagnosis. The question of an acquired abnormality similar to that of hemophilia was considered. The exact nature of the hemophilic defect is disputed. Quick<sup>43</sup> has recently defended the traditional hypothesis that it is a pure thromboplastin deficiency caused by decreased rate of lysis of platelets. This view is perhaps supported by the observation that the prolonged coagulation time of hemophilic blood can be shortened to normal either by the addition of a minute quantity of tissue extract containing thromboplastin, or by the addition of a small amount of normal blood or plasma. On the other hand, the group working at the Thorndike Memorial Laboratory believe that the platelets are normal in hemophilia and that the defect in coagulation is due to the absence of an activator which is present in the globulin fraction of normal blood.<sup>44-47</sup>

We performed a number of experiments to determine if a coagulation defect of the blood similar to that of hemophilia was present in this patient. In Text-Figure 1 are given the results of experiments which were devised to compare the coagulation times of the *whole blood* of the patient with those of a known hemophiliac \* after the addi-

\* The hemophiliac was patient L. T., whose case has been described in detail.<sup>30</sup>

tion of progressively decreasing concentrations of thromboplastin solution.\* A similar series of tests, the results of which are given in Text-Figure 2, was performed in order to compare the *plasma* coagulation times of the patient with those of a hemophiliac after the addition of thromboplastin solutions.†



Text-Figure 1. The effect of progressively decreasing concentrations of thromboplastin solution on the coagulation time of the whole blood of a normal subject, a known hemophiliac, and of the patient W. J. F.

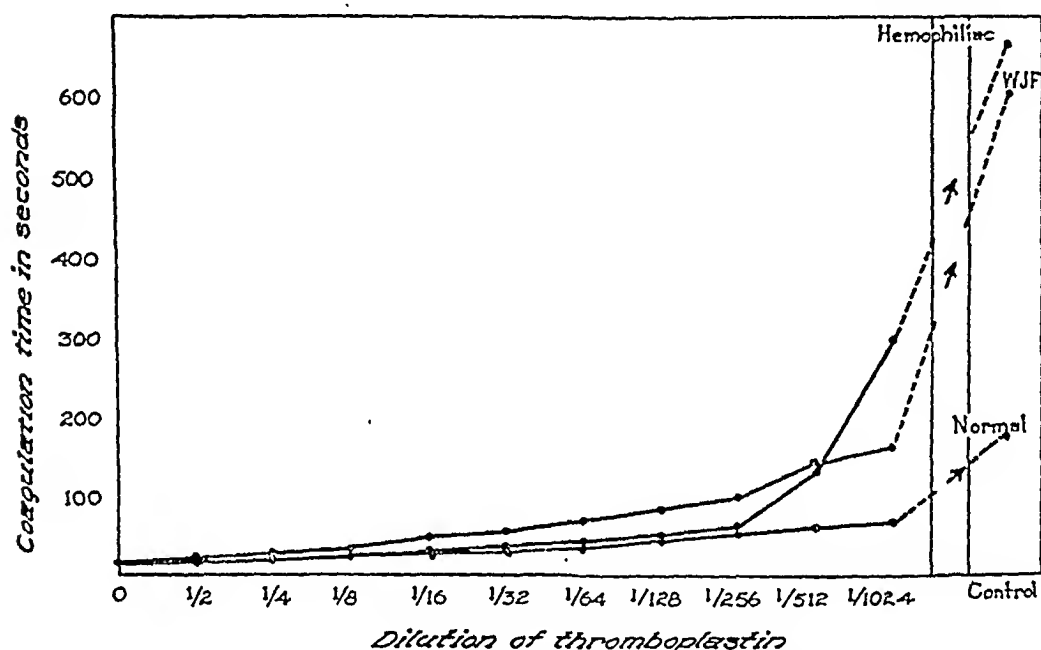
In full strength or in moderate dilution, the thromboplastin solution caused a greater reduction in the coagulation time of the hemophilic blood than of the patient's blood. With greater dilution of the thromboplastin solution there was less effect on the coagulation time of either

\* The thromboplastin solution was prepared as for the prothrombin test according to the method of Quick.<sup>43</sup> The tests were performed at 37° C. in 12 by 100 mm. tubes. 0.1 cc. of progressive dilutions of the thromboplastin solution was added to 2 cc. specimens of blood. The tubes were inverted once for mixing.

† The tests were performed in the same manner as the Quick prothrombin test<sup>44</sup> except, that a series of dilutions of the thromboplastin solution was used for testing.



of the bloods and they approached the control time in which physiologic saline solution was substituted for thromboplastin. Similar results were obtained in the tests performed on the respective plasmas. The lessened coagulative activity of the concentrated thromboplastin solutions on the patient's blood or plasma was probably due to the slightly diminished prothrombin concentration in his blood. On the basis of their reactions to thromboplastin, one would not be able to distinguish between the coagulation defect of this patient and that of a hemophiliac with a similar degree of hypoprothrombinemia. However, in another



Text-Figure 2. The effect of progressively decreasing concentrations of thromboplastin solution on the coagulation time of recalcified plasma of a normal subject, a known hemophiliac, and of the patient W. J. F.

series of experiments in which the coagulation times of the whole blood of the patient were compared to those of the hemophiliac after the addition of graded quantities of normal blood (Table V) and plasma (Table VI), a difference between the two bloods was clearly demonstrated. The same quantities of normal blood or plasma which produced a marked reduction in the coagulation time of hemophilic blood had little effect on the coagulation time of the patient's blood. Furthermore, the transfusion of whole blood did not shorten the coagulation time of the patient's blood in a manner comparable to that usually seen in hemophilia.

Unfortunately, with the present available methods, it is impossible to measure the thromboplastin content of human blood, and therefore one cannot prove that a condition of hypothromboplastinemia exists. It seems reasonable to assume that it is present, however, when, as in

the present case, (1) there is a significant degree of thrombocytopenia, (2) the structure of the platelets is altered, (3) the coagulation defect is corrected by the addition of thromboplastin, and (4) all other known causes of delayed blood coagulation have been excluded.

TABLE V

*Coagulation Times of the Blood of W. J. F. and of a Known Hemophiliac before and after the Addition of Graded Quantities of Normal Human Blood*

Added citrated normal blood	Coagulation time of 2 cc. blood specimens at 37°C.	
	Hemophiliac	W. J. F.
(cc.)	(minutes)	(minutes)
0 (control)	120	23.5
0.02	24	17
0.05	19.5	16
0.10	13	19
0.20	11	20

If it is assumed that the cause of the delayed coagulation time in this patient was hypothromboplastinemia due to deficient platelet numbers and function, then the results of these experiments would tend to support the belief of the Thorndike group that the substance in normal blood which is effective in shortening the coagulation time of hemophilic blood is not thromboplastin but some other plasma constituent.

### *The Thrombotic Features*

Thrombosis may be caused by alteration in the diameter of the vessel lumen with consequent changes in the blood current, by damage to the intima of blood vessels, and by physicochemical changes in the blood. Alterations in the blood itself, which, under certain circumstances, may contribute to the formation of thrombi, include increases in the number or agglutinating power of the platelets, the coagulability of the blood, the quantity of the globulin-fibrinogen fraction of the blood proteins, and increased liberation of thromboplastin from disintegrated blood and tissue cells.

TABLE VI

*Coagulation Times of the Blood of W. J. F. and of a Known Hemophiliac before and after the Addition of Graded Quantities of Normal Human Plasma*

Added citrated normal human plasma	Coagulation time of 2 cc. blood specimens at 37°C.	
	Hemophiliac	W. J. F.
(cc.)	(minutes)	(minutes)
0.0 control	120	25
0.02	25	19
0.05	15	20.5
0.10	14.5	22
0.20	15	25

Despite the presence of a marked hemorrhagic tendency, the patient developed thrombosis in the cavernous and basilar sinuses, in the veins of the arms, and in the laryngeal submucosa. It is difficult to determine the cause of these thrombotic episodes. Because of the marked bleeding tendency, it seems logical to assume that spontaneous hemorrhages into the septa of the cavernous sinuses resulted in narrowing of the lumina, producing endothelial damage with consequent thrombosis. The partial thrombosis of the basilar sinuses may have been caused by propagation from the cavernous sinuses. On the other hand, the original thrombosis may have occurred in the nasal tract following treatment of epistaxis, and may have been propagated thence to the cavernous sinuses.

The thrombi noted in the veins of the submucosa of the larynx were probably secondary to a large hemorrhage which had occurred several weeks before death. Phlebothrombosis in the arms occurred only along the course of veins traumatized by venipuncture. No primary alteration in the endothelium of arteries or veins could be detected in any of the material studied.

Although the primary cause of thrombosis is not discoverable in this case, the laboratory data give evidence that the blood platelets failed to function properly in the process of blood clot retraction; <sup>48,49</sup> they failed to assist in maintaining capillary continuity; <sup>5</sup> and they did not supply enough thromboplastin to ensure an efficient coagulation of the blood.

#### SUMMARY

A case of primary thrombocytopenic purpura is presented, in which there was a markedly prolonged coagulation time of the blood in addition to the usual findings of prolonged bleeding time, diminished blood clot retraction, and increased capillary fragility. The patient had had frequent epistaxis and had bruised easily since childhood, and for 1 year before death these symptoms had increased in frequency and severity and were associated with the appearance of numerous petechial hemorrhages on the lower extremities. While in the hospital the patient suffered from a generalized purpuric eruption, epistaxis, retinal hemorrhages, bleeding into the oral and pharyngeal mucous membranes, bleeding hemorrhoids, bleeding into the right maxillary sinus, subpleural hemorrhages, a large submucous hemorrhage into the posterior wall of the pharynx, gross hematuria, and a large meningeal hemorrhage. Terminally, he presented the signs of bilateral cavernous sinus thrombosis.

The post-mortem examination disclosed: (1) Hemorrhages in the subarachnoid space; in the fibrous septa, nerves and ganglia of the

cavernous sinuses; and in the mediastinum, lungs, subpleural space, right auricle, kidneys, stomach, adrenal gland, vocal cord, scalp, and skin. (2) Thrombosis of the basilar and cavernous sinuses, and of veins in the arms and laryngeal submucosa. (3) Generalized arteriosclerosis with atherosclerosis of coronary arteries and aorta, and hyperplastic arteriosclerosis involving small arteries of the myocardium, lungs, gallbladder, and of the capsules of the pituitary and adrenal glands.

The prolonged coagulation time was thought to be due to hypothyromboplastinemia caused by deficient platelet numbers and function because: (1) there was a significant degree of thrombocytopenia, (2) the structure of the platelets was altered, (3) the coagulation defect was corrected by the addition of thromboplastin, and (4) all other known causes of delayed blood coagulation were eliminated. The coagulation defect differed from that found in hemophilia in that it was not corrected by the addition of small amounts of normal blood or plasma *in vitro* nor by the transfusion of whole blood *in vivo*.

The cavernous sinus thrombosis appeared to be due either to propagation of a thrombus from the nasal submucosa, or to spontaneous hemorrhages into the septa of the cavernous sinuses with resulting endothelial damage and narrowing of the lumina.

We wish to thank Joan Howard Hudson for the statistical analyses.

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[ Illustrations follow ]

## DESCRIPTION OF PLATES

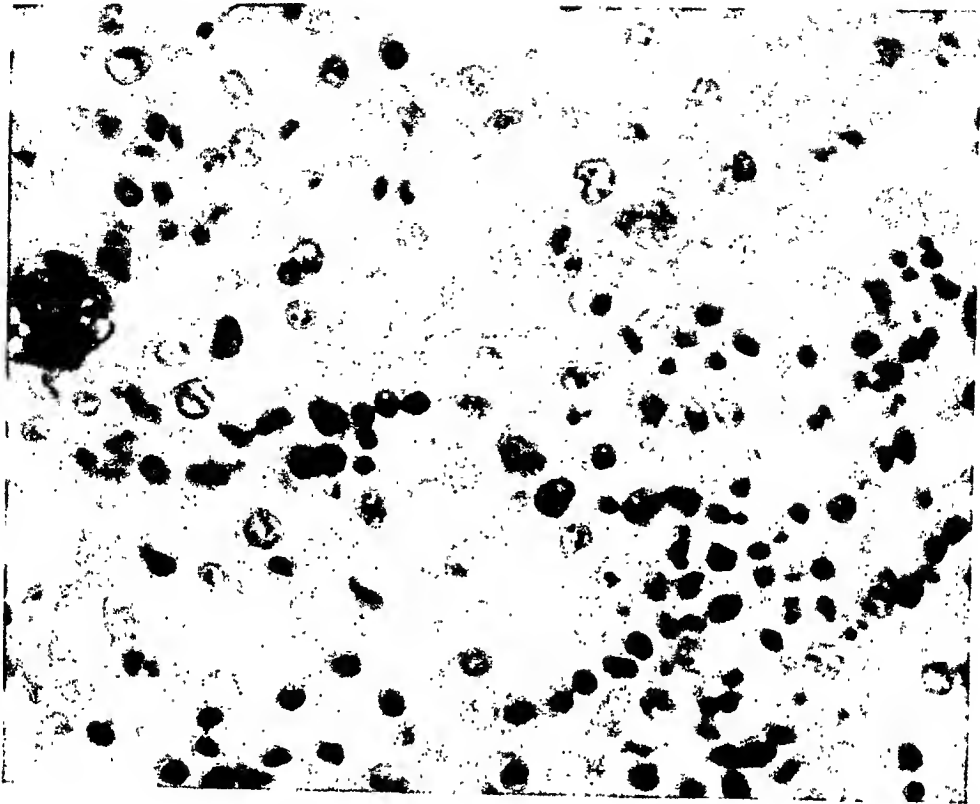
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### PLATE 239

FIG. 1. Bone marrow of lumbar spine.  $\times 650$ .

FIG. 2. Cavernous sinus showing thrombosis with organization.  $\times 120$ .

1



2



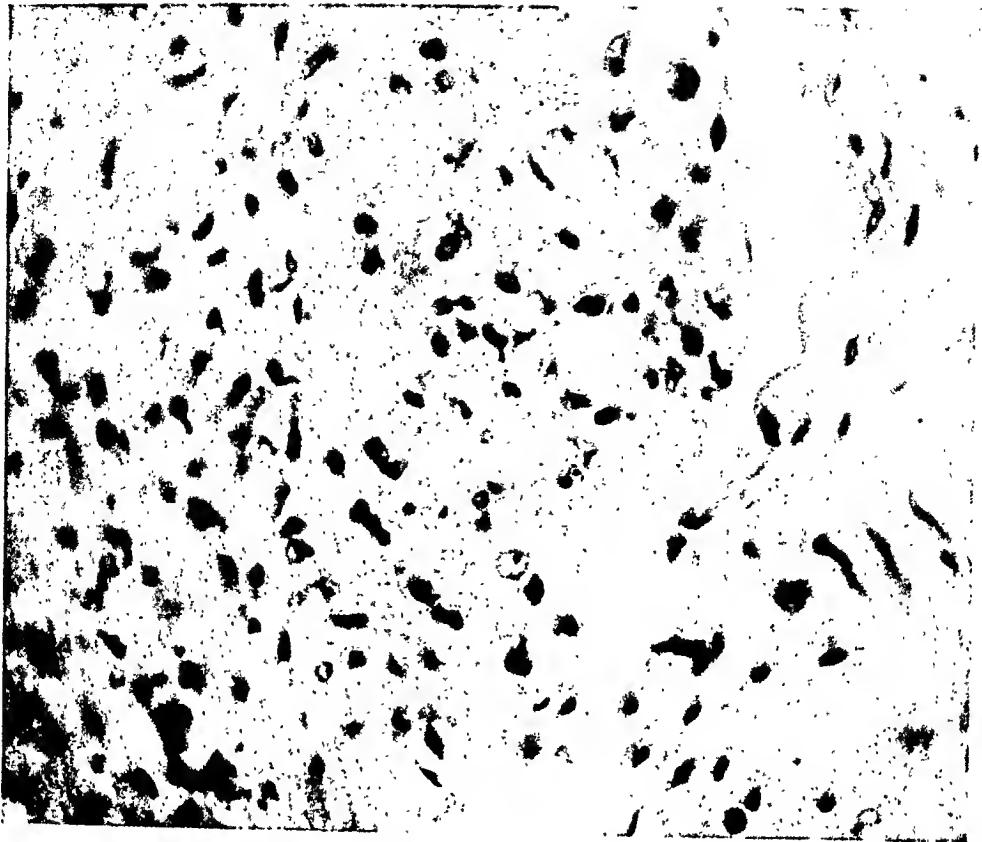


PLATE 240

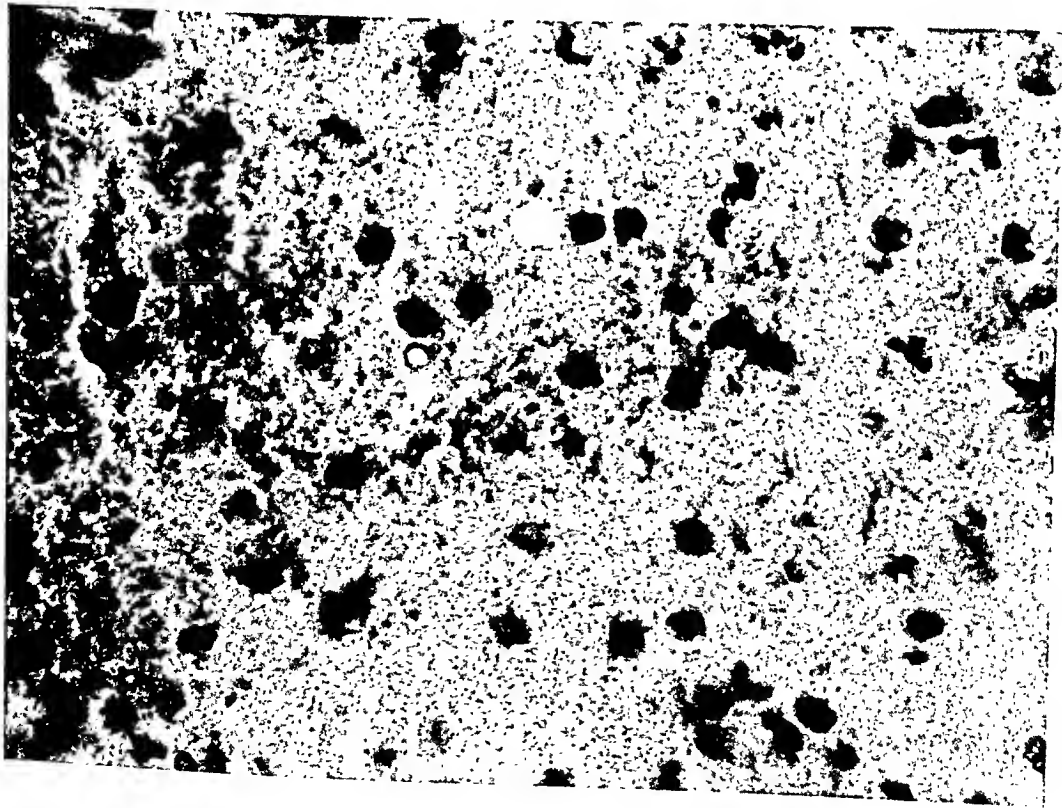
FIG. 3. Higher magnification of one area shown in Figure 2. Late organization of the thrombus.  $\times 500$ .

FIG. 4. Portion of thrombus from cavernous sinus showing occasional leukocytes and erythrocytes, separated by granular masses of platelets.  $\times 750$ .

3



4



Aggeler, Lindsay, and Lucia

Coagulation Defect in Thrombocytopenic Purpura



# STUDIES ON AMEBOID MOTION AND SECRETION OF MOTOR END-PLATES

## VIII. EXPERIMENTAL MORPHOLOGIC PATHOLOGY OF THE CHEMICAL TRANSMITTER OF NERVE IMPULSES IN THE COURSE OF WALLERIAN DEGENERATION \*

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The effects of nerve impulses on the structure of muscle are unknown. The morphologic effects of wallerian degeneration, revealed by the gold-and-teasing method of whole muscle fibers, upon the voluntary neuromuscular apparatus, are likewise practically unknown. It is emphasized at the outset of this paper that the value of the observations to be presented depends largely upon the histologic method selected to demonstrate the structural union of nerve and muscle. Objective evidence of the morphologic transmitters of nerve impulses has not been conclusively demonstrated. Presumptive evidence has been presented that the motor end-plates influence the structure<sup>1</sup> of, and that they discharge granules of acetylcholine<sup>2</sup> into, the myoplasm of striated muscle during the early stages of poliomyelitis, certain other acute infections, rigor mortis, injection of lactic acid locally into the muscle, shock, and specific drug actions. There appears to be a metabolic destruction of the motor end-plates under certain nosologic conditions.

The whole theory of the chemical transmission of nerve impulses was the result of a simple and classical experiment by Loewi<sup>3</sup> on the heart of the frog. He established that, upon stimulation of the vagus nerve, acetylcholine appeared in Ringer's solution placed within the cavity of the heart. When this fluid from the donor heart was placed in the cavity of the denervated recipient heart, the inhibition was comparable to that of vagus stimulation. The accelerator nerves were stimulated, and evidence was obtained that an accelerator substance, adrenalin, appeared in the perfusion fluid. Loewi established the fact that a nerve impulse is transmitted to cardiac tissues indirectly by chemical substances which were called chemical transmitters.

When normal blood is circulating through the heart there is rapid disappearance of acetylcholine. This was proved to be due to a hydro-

\* This study was aided by grants from the National Foundation for Infantile Paralysis, Inc., the Baruch Committee on Physical Medicine, and the Marquette University School of Medicine.

Presented in part before The American College of Physicians, November 5, 1945, Chicago, Illinois.

Received for publication, December 18, 1945.

lyzing enzyme in the blood named cholinesterase. This enzyme splits acetylcholine and renders it ineffective. It was likewise proved by Loewi<sup>3</sup> that eserine has an inhibitory effect on cholinesterase. That eserine prolonged the effect of vagus stimulation had been known for a long time.

Dale, Feldberg, and Vogt,<sup>4</sup> after indirect stimulation through the nerve fibers of perfused voluntary muscles, found acetylcholine in the eserinated perfusion fluid. They stated that artificial perfusion with an eserine-containing saline fluid was necessary for success and that all that their evidence showed was that acetylcholine, which had escaped from the sites of its liberation, required protection by eserine to enable it to diffuse into fluid perfused through the vessels. Curare was demonstrated to have an effect on the receptive element by making the muscle substance more resistant to the action of acetylcholine. They likewise established the fact that direct stimulation of muscle tissue 10 days after denervation did not release a trace of acetylcholine. This substance, therefore, was released from the nerve endings and was not a product of muscle activity or of the blood supply. Brown, Dale, and Feldberg<sup>5</sup> then demonstrated that acetylcholine injected into the artery close to the bled muscle caused a rapid muscle twitch similar to that produced by nervous stimulation.

Nachmansohn<sup>6</sup> demonstrated subsequently that cholinesterase was more highly concentrated about the nerve endings in the sartorius muscle of the frog than at the ends of the muscle. Nachmansohn and John<sup>7</sup> found choline acetylase in the nerve axon, and stated that the presence of this enzyme is consistent with the view that the primary event responsible for the alteration of the axon membrane during the passage of the nerve impulse is the release and removal of acetylcholine, and that the energy-rich phosphate bonds during recovery are used for the synthesis of the acetylcholine removed during activity. It was found that only a part of the initial enzyme activity had been lost 3 days after nerve section when conductivity had disappeared.

The relationship of the production of acetylcholine and its destruction to the pleomorphism of the motor end-plates and the various fiber types has not been established. It is known that differences among the fibers disappear after starvation, denervation, muscle contraction, and heat rigor. The mechanism of production of dark and light fibers in the same mammalian muscle is still obscure.

Waller<sup>8</sup> was the brilliant initiator of the studies in the degeneration of nerves almost 100 years ago, yet wallerian degeneration of the neuromuscular apparatus in voluntary muscle has never been followed in a closely graded series of changes by the gold-and-teasing method of whole muscle fibers following the sectioning of the motor nerves. Waller

made his observations primarily on fresh tissue and on tissue treated with caustic potash. Huber<sup>9</sup> used methylene blue in his experimental studies and cited the limitations of this method. Cajal<sup>10</sup> used the silver and sectioning method which likewise has its limitations. The advantages and disadvantages of the gold method will be cited under "Materials and Methods" in this paper.

The precocious failure of transmission of nerve impulses after the third day in the course of wallerian degeneration of cholinergic nerve fibers has been attributed recently (a) to an early degeneration of nerve endings (Titeca<sup>11</sup>); (b) to a decrease of acetylcholine liberation (Coppée and Bacq<sup>12</sup>); (c) to heterochronism (Chauchard<sup>13</sup>); and (d) to a failure of conduction in the nerve fibers (Rogers and Parrack<sup>14</sup>). The contradictions of fact and of inference involved in these four interpretations prompted a restudy of the problem by Lissák, Dempsey, and Rosenblueth,<sup>15</sup> who came to the conclusion that the mechanism of liberation of acetylcholine at cholinergic nerve endings upon the arrival of a nerve impulse is unknown and that the failure of transmission of nerve impulses about the third day after denervation is due to a progressive decrease in acetylcholine liberation, thus substantiating the theory of Coppée and Bacq. Lissák, Dempsey, and Rosenblueth concluded that total failure of transmission about the third day after nerve section was only a terminal step in a gradual decaying process and that this failure of transmission preceded the sudden failure of conduction in the distal stump of the cut nerve.

One great gap in our knowledge of the transmission of nerve impulses through motor end-plates is due to the absence of conclusive evidence based on experimental morphology. The chemical and physiologic actions in the body are intimately related to the structural arrangement of the protoplasm. If the motor end-plates are microscopic endocrine glands that discharge, under exaggerated stimulation, neurogenic bodies or neurosomes composed of acetylcholine, then microscopic morphology must substantiate this claim.

In this paper we have occupied ourselves with the exact and clear description of those morphologic facts which are certain and easily verified. This has necessitated a large number of illustrations in the form of untouched photomicrographs made from unimpeachable preparations. This gives an atlas of the sequence of changes during the course of wallerian degeneration of the motor end-plates which may serve as a source of reference and a line of departure for additional experimental studies and for the basis of the hypothesis that ameboid motion occurs at these motor end-plates and that a granular secretion is discharged into the muscle.

The objectives of this paper, therefore, are the morphologic demon-

stration of untouched photomicrographs which substantiate the following statements: (1) that there is a morphologic as well as a chemical transmitter of normal and abnormal nerve impulses; (2) that the motor end-plates under normal conditions discharge periodically a finely granular secretion which forms a nebulous rhythmic wave of diffusion into the myoplasm, and which may be agglutinated into large neurosomes in the course of wallerian degeneration; (3) that these motor end-plates undergo a periodic retraction and expansion by ameboid motion in relation to the storage in them, and the discharge from them into the myoplasm, of neurogenic substances; (4) that the failure of transmission of nerve impulses into the muscle about the third day after nerve section parallels the disappearance of the discharge and diffusion of the fine neurogenic granular secretion and the appearance of pathologic changes in the neuromuscular apparatus; (5) that the functional, dark type of muscle fiber, which progressively disappears after denervation, is normally associated with the periodic discharge and diffusion of the fine neurogenic granules of acetylcholine from the motor end-plate into the myoplasm at the onset of contraction; (6) that the same muscle fiber may be either dark and granular or light and relatively agranular dependent upon the functional phases of either acetylcholine diffusion, at the onset of contraction, or acetylcholine destruction by cholinesterase during full contraction, respectively, when the fibers were fixed during fractional contraction; (7) that the increase of the granules around the bloated subsarcolemmal nuclei is coincident in time with the persistence of cholinesterase after denervation; (8) that the facts support the claim that denervation atrophy of muscle is associated with the loss of the normal periodic diffusion from the motor nerve endings into myoplasm of acetylcholine granules having a strong affinity for gold, and that, conversely, the hypertrophy of normal muscle due to the chronic effect of exercise may be the result of the quantitative increase, over a unit of time, of the discharge of neurogenic granules from nerve endings to muscle; and (9) that the relationship of nerve to muscle is one of periodic anatomic continuity through the confluence and compounding of neurogenic and myogenic substances. The structure of voluntary muscle, therefore, has a dual composition.

#### MATERIALS AND METHODS

One hundred adult white rats (*Mus norvegicus*), 10 to 12 months old, with an average weight of 255 gm., were used. All surgical procedures were carried out under ether anesthesia and aseptic precautions. Segments 1 to 3 cm. in length were excised from the right sciatic nerve at the level of the trochanter. The left sciatic nerve remained intact and the neuromuscular apparatus of the left gastrocne-

mius muscle was used as a control. Since our observations were confined largely to the first 30 days after section of the right sciatic nerve, our experiments were not complicated by regeneration, as was proved by lack of response of the muscle to electric stimulation of the distal stump of the nerve. The reaction of the gastrocnemius-soleus muscle to indirect faradic stimulation was tested through the distal stump of the cut sciatic nerve immediately after the operation and on the dates selected for excision of the muscle. There was progressive decrease in the response of the muscle to stimulation of the nerve until the third to the fifth day, after which nerve stimulation was ineffective in producing muscle contraction.

In preparing the muscle for the gold technic previously described,<sup>1</sup> the animals were placed under light ether anesthesia and the gastrocnemius muscles quickly excised. This procedure avoided the changes that might occur with post-mortem rigidity.

At designated times after nerve section some animals were curarized, by either intramuscular or intraperitoneal injection of approximately 1 mg. per kg. of d-tubocurarine chloride (Squibb), until neuromuscular block occurred. Under light ether anesthesia the sciatic nerve and gastrocnemius muscle were exposed on the left and right side. In some, the sciatic nerves on both sides were stimulated at the rate of 5 to 10 times per second for 30 seconds with the double electrodes composed of nickel-plated copper, of the Dumont variable frequency stimulator type 210.

After the operation, the gastrocnemius muscles from 5 rats were excised after each 24-hour interval up to 15 days. The muscles from 2 rats were excised daily from the 15th to the 25th day after nerve section, and those from the remaining 5 rats were excised on the 30th day.

The excised muscles were subjected to various histologic methods for the identification of lipoids and other substances and to visualize the nuclear and cytoplasmic constituents of the neuromuscular apparatus. The Cajal<sup>10</sup> silver method, as well as that of Bielschowsky modified by Boeke,<sup>16</sup> is a good one to identify nuclei, neurofibrils, and the periterminal network of Boeke. Since sectioning obscures the whole structure of the neuromuscular apparatus, and since the chemicals used are found to alter the real structure of the union of nerve and muscle, the silver method should be checked against results obtained by methylene blue and gold. Murray<sup>17</sup> stated that the heavy treatment of tissues with formalin and other chemicals in the silver method is bound to cause serious shrinkage effects and that the process is of such capricious character that tissues treated in exactly the same way show different results.

Huber,<sup>9</sup> in his observations on the degeneration and regeneration of



motor and sensory nerve endings in voluntary muscle, used methylene blue. He stated that, due to limitations of methylene blue, the changes in the nerve endings were not revealed beyond the second day after nerve section, and that the precariousness of the method is such that in normal tissue nerve fibers and nerve endings remain now and again wholly unstained or are only partially brought to view.

The inconstancy of Ranvier's gold method is pointed out by Galigher<sup>18</sup> as follows:

"Unfortunately the formation of gold deposits upon the structures is brought about by an exceedingly delicate reaction which is not well understood, and cannot be obtained with any degree of certainty. The method is notoriously unreliable, and several trials must often be made before a satisfactory impregnation is obtained. However, the results are sufficiently beautiful to justify the effort required to obtain them. It is regrettably true that for nearly fifty years no effort has been made to improve the method in this respect."

We<sup>1,2</sup> have succeeded in obtaining consistent results with our modification of the gold method. Since gold chloride forms *in vitro* periodic precipitates of the Liesegang type with acetylcholine, choline, lecithin, cholesterol, and certain other lipoidal substances, comparable to those cross striations of capillary chemistry previously published,<sup>1</sup> we are inclined to attribute a specific reaction between gold and the normal and abnormal axonic transmitters. The axonic substance is the part that dominantly reacts to gold, but under certain conditions the products of metabolism or breakdown of myelin likewise combine with gold. In the chemical detection and quantitative analysis of acetylcholine and choline by Loach,<sup>19</sup> gold chloride is used. The resulting compound is either acetylcholine aurichloride or choline aurichloride.

The striking neuromuscular changes demonstrated by the gold-and-teasing method will constitute the anatomic basis of this report. The one limitation is the lack of clear visibility of the nuclei. This may be revealed by the observation of neighboring tissues with nuclear stains and with silver. By the use of multiple neurologic methods, confidence is gained regarding the true structure of the union of nerve and muscle, and of the advantages of each method.

The experimental and control muscles were run through the identical fluids for the same periods of time.

The detection of the granular and agranular muscle fibers was also made by the study of fresh tissue in physiologic salt solution at 37°C., according to the method of Denny-Brown<sup>20</sup> and Hines.<sup>21</sup>

The success of our modification of the gold-and-teasing method for whole muscle fibers appeared to be due to the following factors: (1) the initial acidulation of the finely cut fresh muscle with lemon juice or

citric acid, which fixed either acetylcholine or choline: the strong bases, choline and acetylcholine, are stabilized by this initial treatment with acid; (2) the gold chloride, which appears to have a selective chemical affinity for the normal axon and its secretory discharge into muscle, and for certain abnormal products in the myelin; (3) the reduction of the gold by the use of formic acid, which stabilized the normal and abnormal nerve products discharged by the hypolemmal axons into the denervated myoplasm; and (4) the retention of the anatomic continuity of the relationship of nerve and muscle by the teasing of whole muscle fibers and nerves. The continuity of long stretches of the epilemmal axon, hypolemmal axon, sarcolemma, the granules of the sole plate of Kühne, and the cross striations of the muscle fiber are preserved and may be studied in one field of microscopic observation. This relationship is obscured by sectioning muscle fibers after silver impregnation. The granules of Kühne are not stained by methylene blue. Dark muscle fibers have a strong affinity for gold and are dark red, purple, or blue: in the light fibers these colors are decreased or absent in teased specimens.

## RESULTS: EXPERIMENTAL MORPHOLOGY

### 1. *The Pleomorphism of the Normal Motor End-Plates in Relation to Dark and Light Voluntary Muscle Fibers*

The dark and light muscle fibers were clearly evident in the normal control gastrocnemius muscles after the gold technic (Figs. 1, 10, 22, 34, and 35). The dark muscle fibers had more material with an affinity for gold than the light muscle fibers. The dark anisotropic, transverse bands in the dark muscle fibers were usually broader and darker than those in the light fibers. On the other hand, the light, isotropic, transverse striations in the light muscle fibers were usually not only broader than the corresponding light spaces in the dark muscle fibers, but there was less material that had an affinity for gold in the light spaces of the light muscle fibers than in those of the dark fibers.

The width of the muscle fiber was a variable factor depending upon fixation in a state of either isometric and isotonic contraction or the termination of relaxation at the onset of contraction. Some light muscle fibers, therefore, were smaller in diameter than the dark ones. The variable capacity of the different muscle fibers for gold impregnation was just as reliable as, and more permanent than, the study of fresh teased muscle. The dark muscle fibers may be classified as hyperchrysophilous and the light ones as hypochrysophilous. The cross striations

in dark fibers are composed of neurogenic and myogenic substances. There were multiple gradations in the affinity of the fibers for gold between the two extremes. The fading of the striations in denervated muscle produced fibers that may be classified as achrysophilous.

The motor end-plates in the dark and granular muscle fibers usually possessed coarse fronds or knob-like terminals. Some of these fronds were surrounded by a light halo-like space. The retracted motor end-plates were usually surrounded by more dark granular material of the sole plate of Kühne than those of the relatively expanded end-plates in the light muscle fibers. There appeared to be a direct continuity in some places between the granules of the sole plate of Kühne and those condensed in the periodic dark cross striations of the myoplasm.

The granules of the sole plate of Kühne appeared to be derived from two sources; namely, from the granular transformation and permeability of the terminals of the hypolemmal axons of the motor end-plate, and from the nuclei of the sole plate. This granular material of the sole plate normally appeared to diffuse in a periodic manner throughout the myoplasm of the dark muscle fiber. The structure of the motor end-plate in the light muscle fiber was usually one in which there was an ameboid expansion and an attenuation of the hypolemmal axons of the motor end-plate. There was, likewise, a decreased amount of the diffusible granules of the sole plate of Kühne around these relatively expanded motor end-plates of the light muscle fiber. Because the dark muscle fiber had a strong affinity for gold and this quality was gradually lost by denervation, it was compared to the stage of periodic discharge and diffusion of acetylcholine from the motor end-plate into the myoplasm. The dark muscle fiber was correlated with the termination of relaxation, or the onset of muscle contraction, and the light one with the phase of active, full contraction, in the fractional contraction of the muscle as a whole. This fractional contraction is lost progressively by denervation and its loss is correlated with the loss of the phase of differential diffusion of acetylcholine into the muscle. It has been practically impossible to catch a muscle fiber in the state of completely unstable and physiologic relaxation by any histologic technic used to date. The dark muscle fiber probably represents the structure nearest to that of relaxation, or the onset of contraction of the myoplasm produced by the periodic diffusion of acetylcholine.

The decreased visibility of the cross striations at the onset of contraction, observed in living muscles by many investigators, may represent this stage of the periodic discharge and diffusion of acetylcholine. The periodic structures in the muscle fiber would then be obscured, altered, and realigned in a rhythmic manner by the capillary chemical changes in the metabolism of the neuromyoplasm. The shuttle-like

shift of the cross striations described by Jordan<sup>22</sup> could easily be explained on the above basis.

## *2. The Progressive Loss of the Dark Voluntary Muscle Fibers after Denervation*

There was a progressive loss of muscle fibers of the dark type beginning 24 to 48 hours after denervation. There was an agglutination of the fine granular material that had an affinity for gold into coarse clumps, which gave a flaky appearance to the myoplasm of the muscle fiber (Figs. 6 and 7). This was a histologic sign of the beginning loss of the normal nebulous diffusion in a periodic manner of the granular substance discharged from the denervated motor end-plate. This was microscopic evidence of the beginning dissociation of the nerve and muscle substances in the muscle fiber, characterized by the initial changes in the segregation and accumulation into larger aggregates of the normally fine neurogenic granules. It was a sign of the beginning loss of substantial influence of the innervation upon the muscle. A search is now in progress to produce the chemical denervation of muscle by the injection of some substance that will combine with the chemical transmitter of nerve impulses and thereby inactivate, through segregation, the neurogenic from the myogenic substances in the muscle fiber. Preliminary experiments point to the fact that DDT can play this rôle.

There was also histologic evidence of the loss of the normal fractional contraction of the muscle fibers after denervation. All of the motor end-plates in a specific field were either abnormally retracted with an increased affinity for gold, or were expanded and decreased in their capacity to take the gold (Figs. 4 to 9). On the third day, there were certain fields within the denervated muscle that were totally devoid of muscle fibers of the dark type (Figs. 2 and 11). From the fifth to the tenth day (Figs. 12 to 16) practically all of the dark type had progressively disappeared. The dark muscle fiber had coarse, irregular, longitudinal myofibrillae, whereas those in the light muscle fiber were smaller in diameter and more regularly arranged. The arrangement of the myofibrillae appeared to be correlated with the diffusion and disappearance of the fine granular material that had an affinity for gold. The accumulation of this granular material characterized the dark muscle fiber that was usually narrow and coarsely striated in a longitudinal direction, whereas the light muscle fiber, characterized by the disappearance of the granules with an affinity for gold, was usually wide and composed of fine fibrils arranged longitudinally. The accumulation and depletion of the chrysophilous granules appeared to be correlated with the functional activities of the muscle fiber, based on the diffusion and hydrolysis of the granules of acetylcholine.

### 3. *The Progressive Pathologic Changes of the Motor End-Plates after Denervation*

During the first 48 hours (Figs. 4 to 9, and 36 to 49) after nerve section, there were relatively normal motor end-plates scattered among those undergoing structural changes. There were noticeable structural changes of the end-plates and adjacent epilemmal axons within the first 24 hours. These were detected by beginning enlargement of about 10 per cent of the epilemmal axons and the formation of retention cysts on the ramifications of some retracted hypolemmal axons of the motor end-plates (Figs. 5, 36, and 37). This retraction, increased affinity for gold, and cystic enlargement of the branches of the nerve endings became structural characteristics in certain microscopic fields from 24 to 48 hours after nerve section (Figs. 6, 9, 41, and 42). Around some retracted nerve endings there was evidence of the granular sole of Kühne, whereas these granules were absent around other retracted nerve endings. The granular sole was most frequently absent around the expanded endings composed of elongated and attenuated branches (Figs. 7, 40, and 45).

In other fields the nerve endings were expanded, some had decreased affinity for gold, and their ramifications were exceedingly attenuated (Figs. 40, 45, and 49). This uniformity of retraction of the nerve endings on certain trees of innervation, and the expansion of all of the nerve endings on others, were histologic evidences of loss of the normal pleomorphism usually found on each tree. They are likewise histologic signs of loss of the normal fractional contraction of the muscle fibers under the influence of normal innervation. This was evidence of uniform overstimulation and overexcitation of the motor units innervating the denervated muscle after nerve section. Between 5 and 10 per cent of the nerve endings were replaced by fine granules at 48 hours. This replacement was found in about 60 per cent of the nerve endings at 72 hours. These figures are based on a differential count of 5,000 motor end-plates.

Concomitant with the structural changes of the motor end-plates, there were associated alterations in the structure of the muscle fiber. Coincident with the loss of the normal periodic diffusion of fine chrysophilous granules transmitted from the motor end-plate, there was seen a coarse flakiness of these granules in the muscle fibers. In some trees of innervation the epilemmal axons were fragmented and had a decreased affinity for gold (Fig. 8), whereas in others there was an increased capacity to take the gold with little evidence of fragmentation (Fig. 9). Since these observations were made in the same muscle and since the technic was standardized by running the normal and abnormal

muscles simultaneously and similarly, this was considered a histologic sign of periodic decrease alternating with increase in the quantity of the gold-impregnated material in the epilemmal axons. There was a definite hyperemia of the intramuscular blood vessels and capillaries (Figs. 8 and 9), demonstrated by the increased diameter of the lumen and the packing of these vessels with red blood cells. In other locations the capillaries were collapsed and devoid of cellular elements. This was comparable to the experimental structural conditions produced by poliomyelitis, traumatic and thermal shock, and by the experimental injection of lactic and other acids locally into the muscle.

The depletion of many of the epilemmal axonic trees of their motor end-plates after the third day subsequent to the degenerative cut of the sciatic nerve was structurally associated with the progressive loss of muscle fibers of the dark type (Fig. 2). There was likewise a distinct fading of the dark anisotropic cross striations due to the loss of granules with an affinity for gold. This was due to the depletion from these striations of fine chrysophilous granules that were normally periodically discharged from the motor end-plates and that diffused into the myoplasm. It was imperative, therefore, that frequent comparisons be made of the normal reaction with gold (Figs. 1, 10, 22, 34, and 35) and the loss of this reaction in denervated muscle.

Although it was true that only the axons of the nerve fibers and their end arborizations were dominantly impregnated with gold, there was no difficulty in differentiating medullated nerve fibers with the faint outline of a sheath and the locations of the nodes of Ranvier from the varicose nonmedullated nerve fibers. In the degenerating medullated nerve fiber, the myelin was impregnated more readily than in the normal one, so that the segments of a degenerated medullated sheath may be made out during the early stages, 48 to 72 hours after nerve section. There was distinct segmentation of the myelin during this time, and the degenerated products fused with those in the central axon. It was clearly evident that the extreme distal end of a severed motor nerve and its motor end-plate degenerate before the remaining portions of the same nerve distal to the point of the degenerative cut. The degenerative changes which follow the segmentation of the myelin and the formation of cystic enlargements on the motor end-plates were clearly followed by the gold-and-teasing method applied to whole muscle fibers. Huber<sup>9</sup> stated definitely that it was impossible for him to follow the changes with the methylene blue technic the second day after the section of the nerve. The granular degeneration was well advanced in the motor end-plates 72 hours after denervation (Figs. 50 to 55).

Sprays of medullated nerve fibers underwent progressive degeneration by the depletion and discharge of their degenerated contents, in a centrifugal direction, from 5 to 30 days after nerve section (Figs. 12 to 21, 56, 57, 69, and 70). The discharged combined axonic and myelin substances had a strong affinity for gold and were accumulated in pleomorphic masses within and around the region of the degenerated motor end-plates. The faint, ghost-like outline of the epilemmal axons depleted of substances that had a strong affinity for gold was in striking contrast to that of the accumulated chrysophilous material discharged in a centrifugal direction into the region of the degenerated motor end-plates. Some of these pathologic masses or neurosomes were found out in the myoplasm of the muscle fiber. The muscle fibers 20 days after the degenerative cut (Fig. 19) had lost the appearance of the normally functioning dark type and were very narrow in comparison with the normal. This anatomic evidence shows conclusively that the histologic dark type of muscle fiber is dependent upon the normal functioning of the nerve supply. This functional dark type of muscle fiber, therefore, appears to be produced by the normal periodic diffusion into the myoplasm of the fine granules of acetylcholine discharged from the motor end-plate.

The axonic material which was discharged in a centrifugal direction 30 days after the degenerative cut (Figs. 20 and 21) was seen in relation to only a few of the degenerated motor end-plates. The locations of the degenerated end-plates were shown dominantly by clusters of sole plate nuclei which were surrounded by the granular material secreted by these nuclei. This granular material had an affinity for gold slightly less than that of the material discharged by the hypolemmal axons. The clusters of nuclei were detected as clear, rounded, or oval spaces surrounded by the dark granules (Fig. 20).

In some places in the same muscle, the myoplasm was aggregated into broad and narrow, dark, transverse bands alternating with light ones (Fig. 21). In the dark bands the cross striations were either exceedingly fine or they were completely absent because of the opacity of the dense aggregation of the myoplasm. The light bands were occupied by cross striations which were wider apart than those in the dark bands. This dark and light transverse banding of the narrow muscle fibers was produced by the slow, irregular fibrillation of the muscle fibers. This incoordinate and ceaseless fibrillation, of slow vermiform activity, of the denervated muscle fibers begins about 72 hours after the degenerative cut of the sciatic nerve. This fibrillation was correlated with the loss of the dark muscle fibers and the loss of the transmission of acetylcholine which, normally, chemically tunes the muscle

fiber to the higher pitch or frequency of fast muscle contractions and to the capacity to respond adequately to high frequency stimulation. The chemical tuning process of muscle was roughly analogous to the mechanical tuning of the strings of a piano or violin for response at a higher frequency than that of strings which are loose and sagging.

#### 4. *The Experimental Exaggeration of the Discharge of Large Neurosomes into Denervated Muscle*

The progressive degeneration of peripheral nerves, clearly described by Parker,<sup>23</sup> was accelerated by the intraperitoneal injection of d-tubocurarine chloride. In some animals, after the muscle was curarized, the distal segment of the cut sciatic nerve was stimulated for 30 seconds, at the rate of 5 per second, with no response of the muscle. This experimental procedure caused a massive accumulation of degenerated nervous substances discharged into the muscle from 5 to 20 days after section of the nerve (Figs. 3, 23 to 33, and 58 to 68). There was complete depletion of the material in the epilemmal axons and an enormous accumulation of this neurogenic material in and around the degenerating motor end-plate, with periodic discharges of pleomorphic neurosomes into the myoplasm. This exhaustion of the axonic spray of its specific substances with strong affinity for gold, and the massive accumulation of these chrysophilous substances at the degenerating end-plate and in the muscle constitute conclusive evidence that there was a substantial transfer of some substance from the degenerating nerve to the denervated muscle. The absence of the normal dark muscle fibers was related to the loss of the normal periodic nebulous diffusion of the granules of the sole plate of Kühne. The agglutination of either the normal or abnormal transmitter substance in a relatively non-diffusible form gave rise to these morphologic changes under the conditions of the experiment. This demonstration of the pathologic structure of the abnormal transmitter substance was produced by acceleration of the discharge and prevention of the normal diffusion of granules into the myoplasm of the muscle fiber. There was great variation in the structure of this abnormal substance transmitted from nerve to muscle (Figs. 3, 23 to 33, and 54 to 68).

The discharge of the degenerated axonic and myelin materials into the peripheral terminal zone of the degenerated motor end-plates had a pleomorphic arrangement; namely, unipolar (Figs. 58 and 59), bipolar (Fig. 61), and multipolar or completely circumferential (Figs. 60 and 62). The chrysophilous material had been discharged into and around the degenerating motor end-plates in a centrifugal direction from the epilemmal axons and there was unimpeachable evidence that



this gold-impregnated material was initially in direct continuity with the structures that innervate the muscle. In one end-plate (Fig. 59) the discharged neurosome was in direct anatomic continuity with the hypolemmal axon of the motor end-plate. It formed what has been designated by some neuroanatomists as an ultraterminal nonmedullated branch of the motor end-plate, ending in the same muscle fiber in an enlarged spherical or oblong terminal. This ultraterminal branch of the motor end-plate, however, was merely the initial phase of the discharge of materials from the nerve terminal with anatomic continuity still maintained. It was the product of the abnormal discharge of substances by abnormal stimulation from the pathologic motor ending, and not a morphologic ending of specific type as claimed by some observers.

When the narrow, dark, and granular muscle fiber was observed in cross section, it was impossible to differentiate and definitely identify the neurosomes in ordinary histologic preparations. Even with lipoidal stains, it was impossible to identify with certainty acetylcholine and choline neurosomes from particles of lecithin and cholesterol. When gold preparations were imbedded in gelatin and cross sectioned with the freezing microtome, there was a striking variation in the size and staining capacity of the gold-impregnated neurogenic granules. The same normal or abnormal muscle tissue may be teased and photographed in its longitudinal projection and then imbedded, cut in transverse sections, and studied in that plane. There was no doubt, therefore, of the intramuscular location of the normal and abnormal neurosomes. At some degenerated nerve terminals, part of the neurogenic substance may be found between fibers after electrical stimulation of the distal stump of the cut nerve.

##### *5. The Increase of Granules around the Bloated Subsarcolemmal Nuclei after Denervation*

The clear, rounded, and oval spaces both within the region of the degenerating end-plate and in the granular cytoplasm of the sole plate of Kühne were occupied by nuclei. The nuclei, close to the degenerating nerve endings (the Telodendrienkern of Boeke), appeared first to enlarge and later to become smaller after nerve section (Figs. 50 and 51), whereas the nuclei in the granular sole (Sohlenkerne of Boeke) and the subsarcolemmal nuclei out in the myoplasm were increased in size, for a considerable period of time, and became surrounded by an increased quantity of granules (Figs. 50 to 53). The bloated subsarcolemmal nuclei were depleted of chromatin and showed loss of nucleoli when specifically stained. These bloated vesicular nuclei became surrounded with a greatly increased quantity of chrysophilous granules concomitant

with the loss of the normal periodic discharge of fine granules from the motor end-plates. The peripheral increase of granulation around the muscle nuclei after denervation was likewise coincident with the unneutralized and persistent activity of cholinesterase found by Nachmansohn and John<sup>7</sup> 3 days after nerve section, when conductivity had disappeared from the axon.

The granular material, that had the capacity to take gold, appeared to be contributed by two sources: the granular transformation of the hypolemmal axons of the motor end-plate, and the nuclei of the sole plate and the subsarcolemmal nuclei of the myoplasm. Pommé and Noël<sup>24</sup> stated that the telesomes or sole plate granules decreased or disappeared in progressive muscular atrophy. Tower<sup>25</sup> stated, however, that these granules had not been studied after experimental denervation. The increased visibility of the nuclei in denervated muscle was probably due to three factors: hypertrophy of the nucleus, accumulation of an increased quantity of granules around the nucleus, and decreased cytoplasm which resulted in crowding together the preexisting nuclei. No evidence was found of nuclear division.

The denervation atrophy caused definite shrinkage of the cytoplasm. Tower<sup>25</sup> defended the opinion that denervation atrophy first attacks the sarcoplasm and at a later date the myofibrillar substance. It was noted, however, that there was no discontinuity of the process of atrophy of the sarcoplasm and the myofibrils that would indicate a shift of the process from one structure to another. Tower concluded, therefore, that the sarcoplasm and myofibrils formed a structural continuum. The chemical evidence indicated that the depletion of substance was in fairly equal proportion for both the sarcoplasm and the myofibrils. There was objective evidence in our experimental study with the gold technic that atrophy of the cytoplasm of the muscle fiber was coincident with failure of discharge of the granules associated with the chemical transmission of the normal nerve impulses. The normal myoplasm, therefore, was proved to be composed of myogenic and neurogenic substances. Atrophy was coincident with the loss of one of these substances, namely, the fine neurogenic granules or neurosomes.

#### *6 The Pleomorphism of the Neurosomes in Denervated Muscle*

The discharged neurosomes (Ns., Figs. 71 to 75) found in the myoplasm were pleomorphic and hyperchromatic for gold in comparison with the faded hypochromatic cross striations of the muscle fibers in which they were found. These neurosomes may be fusiform (Figs. 71 and 72), irregularly oblong with one end tapering (Figs. 73 and 74), arrowheaded in shape, or rounded droplets that vary in size (Fig. 75).

The droplets formed either loose series of single drops widely separated or closely related series of 2 to 10 droplets. The droplets in the middle of a series were usually larger than the terminal ones. Such series of droplets formed irregular fusiform structures separated by clear spaces. The large fusiform neurosomes may have either serrated (Fig. 71) or festooned (Fig. 72) edges. The sharp projections arranged like saw teeth around the edges of the neurosomes may or may not be in direct alignment with the dark cross striations of the muscle fiber. These neurosomes undergo a granular dissolution, and the granules become incorporated into the myoplasm, and are then aligned with the cross striations of the muscle fiber. These neurosomes were periodically discharged from the motor end-plates into the muscle fiber. During degeneration they were more persistent, agglutinated, and less diffusible than when produced by the artificial overstimulation of normal muscle or when produced by neurogenic shock.

#### *7. The Experimental Production of Giant Muscle Fibers after Denervation*

Giant muscle fibers (Gmf., Fig. 76) were found in a few 10-to-14-day denervated muscles after the intraperitoneal injection of d-tubocurarine chloride followed by electrical stimulation of the distal stump of the cut nerve for a duration of 30 seconds at the rate of 5 per second. The diameter of these fibers was 3 or 4 times that of closely related muscle fibers. They were densely impregnated with gold. This represented an abnormal discharge and accumulation of increased quantities of abnormal axonic material in the fiber. The cross striations were seen only at the edges because of the opacity. There was a definite streamlined effect produced by certain large neurosomes (Ns., Fig. 77) upon the cross striations of the muscle fiber. This was detected by the altered arrangement of the cross striations. Certain large muscle fibers (Fig. 78) were not so densely packed with neurosomes as were the giant muscle fibers (Fig. 76).

In some locations the neurosomes were dense and opaque while in others they were light and cross-striated. The dense neurosomes were gradually replaced by granules which eventually became aligned with the cross striations in the muscle fiber.

#### *8. The Intermittent and Progressive Degeneration of the Distal Stump after Section of the Motor Nerve*

The degeneration of the distal stump of the sciatic nerve was tested histologically by the gold-and-teasing method of whole nerve fibers and was proved to be progressive in character and not to take place

simultaneously throughout the whole length of the nerve. The changes in the axis cylinder, medullary sheaths, and motor end-plates were unquestionably initially more advanced at the junction between nerve and muscle than in the same nerve fiber far away from the muscle. There was disappearance of the motor end-plates between the third and fifth days. The degenerated material in the trunk of the nerve advanced, in a progressive manner as well as intermittently, in a centrifugal direction. This, then, resulted in a progressive depletion of the proximal end of the distal stump of the nerve while the terminal of the nerve was discharging the degenerated materials into the muscle. One branch of the sciatic nerve was followed just before its entrance into the gastrocnemius muscle 14 days after sectioning. At this location there was complete depletion of the degenerated axonic and myelin substances that had an affinity for gold (Fig. 79). At a slightly more distal point, the gold-impregnated material was found in variable amounts in axis cylinders which were fragmented into rounded, oval, or fusiform bodies with a strong affinity for gold (Figs. 80 and 81). Just before the nerve gave rise to the branches that directly innervate the muscle, there was again a depletion of the axis cylinders of chrysophilous material (Fig. 82). Only in widely scattered areas were five fusiform bodies found that had an affinity for gold. The epilemmal axons of a cut nerve that innervate the muscle are periodically engorged and depleted of the degenerated and fragmented axonic and myelin materials until structural exhaustion occurs. This rhythmic discharge of degenerated nervous material into the muscle continues, in a progressive and periodic manner and in a centrifugal direction, until most of the degenerated material is discharged centrifugally into the muscle.

## DISCUSSION

### *The Mixture of Red and White Fibers in Voluntary Muscle*

No attempt will be made to review the extensive histologic literature accumulated over the past 100 years on red and white muscle and on the controversial subject of the so-called mixture of red and white fibers in the same voluntary muscle. The excellent recent reviews and observations on this topic may be consulted: Tower,<sup>25</sup> Cobb,<sup>26</sup> Needham,<sup>27</sup> Hines,<sup>28</sup> Hinsey,<sup>29</sup> Fulton,<sup>30</sup> Forbes,<sup>31</sup> Creed, Denny-Brown, Eccles, Liddell, and Sherrington,<sup>32</sup> Wilkinson,<sup>33</sup> Roberts.<sup>34</sup>

Cobb<sup>26</sup> makes the following important statements (page 519):

"One must review the *anatomy of muscle* carefully in order to understand the possible effects of the nerve impulse upon it. Even in this field one is surprised to find indefiniteness, partly because the classification of the different types of muscle is inherently difficult on account of intergradations and transitional forms;

partly because the histology of muscle has often been overlooked in the study of its physiology";

and (page 520):

"It must be emphasized here, however, that the state of knowledge concerning the red and white muscle fibres is at present so confused, even in the field of anatomy, that much work must be done before physiological interpretations can be acceptable."

Almost 60 years ago, Grützner,<sup>35</sup> stated that every muscle contains two specific types of fibers, often intimately mixed: one kind narrow and dark, the other broad and light. He thought that the dark color of the narrow fibers was due to numerous granules and that all narrow muscle fibers, whether pigmented or not, corresponded to the red, slowly contracting muscles of the rabbit, and that the large, light, agranular muscle fibers corresponded to the rapidly moving white muscle. Knoll,<sup>36</sup> and Knoll and Hauer<sup>36</sup> designated the cloudy muscle fibers as "sarkoplasmaeichen" and the clear ones as "sarkoplasmaarmen" or "fibrillenreichen." Krause<sup>37</sup> believed that the different kinds of muscle fibers seen by Grützner and Knoll were dependent upon differences in age: the narrow granular fiber was assumed to be younger than the wide light fiber. Bonhöffer,<sup>38</sup> however, demonstrated that the proportion and distribution of the two types of muscle fibers were the same whatever the age of the frog.

The skeletal muscle may vary in color, not only from species to species, but in the muscles of the same animal. The differences in color of the white meat of the chicken's breast muscle and the deep red of the pectoral muscle of the pigeon or wild duck is very striking. In 1865, Kühne<sup>39</sup> studied the cream-colored and red muscles of the rabbit by spectroscopic methods. He concluded that the deep red muscles had a higher content of myohemoglobin than the light cream-colored muscles.

The structural and functional significance, however, of the narrow, dark, and granular fibers and the wide, light, and relatively agranular fibers found in both the red and white muscles has not been solved to date. It was demonstrated by Schäffer and Licht,<sup>40</sup> Tower,<sup>41</sup> and others that denervation atrophy abolishes the morphologic distinction between the two types of muscle fibers. The mechanism, however, of abolishing this morphologic difference in the two types of muscle fibers found in so-called mixed muscles by denervation was not revealed.

Tower<sup>41</sup> referred to the "large pale" and "small granular" fiber types as follows (page 123):

"What such characteristics may signify for muscle function, difference in contraction rate, in the ability to store specific lipoidal or protein products, does not

lie within the province of this paper to consider. Yet the problem is of prime importance. For not until the conditions are known which gave rise to clear-cut fiber types in one cat and not in a second; not until the distribution of such diverse fibers within the muscle shall be better understood, will it be possible to evaluate small quantitative changes, the possible result of lesions of the sympathetic."

Tower<sup>41</sup> stated that sympathetic denervation did not alter the proportion, distribution, or constitution of the fiber types in voluntary muscle.

It was an error made years ago, that has led to endless confusion, to compare these two histologic and functional types of fibers with the distinct red and white varieties of muscle. In both red and white muscles there are in each muscle dark granular and light agranular fibers. The functional activity of the neuromuscular apparatus is associated with the release, diffusion, and subsequent destruction of acetylcholine, processes which have not been associated, heretofore, with the dark and light muscle fibers in the fractional contraction in the same muscle. In fact, the probable relationship of the Q and J granules of Holmgren,<sup>42</sup> certain liposomes of Albrecht<sup>43</sup> and Bell,<sup>44</sup> interstitial granules of Kölliker,<sup>45</sup> and fine granular neurosomes in muscle fibers has been proposed only recently by us.<sup>46</sup>

Tower,<sup>47</sup> likewise, surmised that the muscle nuclei and sarcoplasm were in some obscure way dependent upon the influence of the nervous system, as is evident in the following statement (page 25):

"In the extrafusal muscle fiber, the aggregation of nuclei around the motor end-plate has always suggested a peculiarly intimate relationship of these to the nervous tissue. But nerve lesion affected not only these nuclei physically associated with the disintegrating nervous tissue, but also all the nuclei the entire length of the fiber. Do all these unconnected and seemingly inactive nuclei form, perhaps with the sarcoplasm, an organization, possibly for conduction within the fiber and for excitation of the contractile mechanism, but an organization dependent on the nervous tissue to a degree incompatible with normal existence after nerve degeneration? This is a most alluring interpretation of the reaction of muscle nuclei, in toto, to denervation."

Denny-Brown<sup>20</sup> stated that slowly contracting fibers were able to store lipoids in the form of liposomes with a resultant granular appearance, while few rapid fibers had this property. Histologic differences were found to disappear on emaciation, but the differences in the speed of contraction remained. Denny-Brown claimed that the dark muscle fiber was related to nutrition. He likewise stated that there were certain light muscle fibers which could not be transformed into the dark granular ones. If the light muscle fibers are the stage of full muscle contraction and hydrolysis of acetylcholine, it would not be surprising to find light fibers even in conditions of overnutrition. Regardless of the method used, a certain number of the granular muscle fibers would

be transformed into the agranular type due to contraction. There would be relatively more fibers of the dark granular type observed in muscles of slow activity in comparison to those with high rate of speed of action, because there would be a correlation between the rate of cycles of diffusion and hydrolysis of acetylcholine granules and that of relaxation and contraction of the muscle fiber. The speed of neuromuscular metabolism would, therefore, tend to determine the relative proportion of fiber types. The irreversible high rate of metabolism to the level of fixation, by heat rigor, is associated with the abnormal overproduction of the light agranular fibers in frog muscle.<sup>1</sup>

The mechanism of production of the atrophy of muscle by denervation has been an obscure problem. This, likewise, applies to the hypertrophy of muscle due to the chronic effects of exercise. Carlson and Johnson<sup>48</sup> (page 369) made the following comments:

"The muscle enlargement with correspondingly greater strength, which is developed by work or training, is a commonly observed phenomenon. There is evidence that the muscle enlargement is not due to any increased number of muscle fibers, as might be suspected, but rather to an increase in size of each fiber. The exact nature of this effect, apparently a growth phenomenon, or the mechanism of its production are not yet known. The effect seems in some way to be related to the influence of nerves and nerve impulses reaching the muscle by way of the efferents. At any rate, if the number of nerve impulses is reduced, as in disuse of the muscle, the muscle fibers become smaller, and the whole muscle shrinks in size and becomes weaker. And, if the efferent nerve of a muscle is destroyed by disease or accident, so that all nerve impulses to the muscle are cut off, the muscle shrinks in size greatly and may entirely disappear and be replaced by fibrous connective tissue, leaving no trace of the former structure."

It is our opinion, supported by experimental morphologic evidence presented in this and other papers, that some light has been shed on the obscure mechanism of the atrophy and hypertrophy of voluntary muscle by the elimination and overproduction, respectively, of a substantial transfer of materials from nerve to muscle. The degree of atrophy and hypertrophy appear to be related to the degree of the decrease and increase of the neurosomes respectively discharged from the motor end-plates into the myoplasm of the muscle fiber. There are unquestionably related vascular changes which are likewise important.

Loewi and Navratil<sup>49</sup> proved that atropine, and Navratil<sup>50</sup> that ergotamine, did not paralyze the respective nerves to the effector organs by showing that after their application nervous excitation is still effective in liberating the transmitters. This was likewise shown to hold for nicotine by Feldberg and Vartiainen,<sup>51</sup> and for curare by Brown and Feldberg<sup>52</sup> and Brinkman and Ruiter.<sup>53</sup> These pharmacologic experiments demonstrate that acetylcholine acts directly upon the voluntary

muscle and that chemical antagonists counteract this effect. The true point of attack of these important drugs is upon the neurogenic component of the compound neuromyoplasm. These drugs alter the structure of the motor end-plates and likewise form agglutinated masses of large neurosomes in the myoplasm. The experimental morphologic findings in this paper support the interpretations of the action of the above drugs on the myoplasm. Dale, Feldberg, and Vogt<sup>4</sup> concluded, likewise, that when transmission of excitation from the nerve to the perfused muscle is prevented by curarine, stimulation of the motor nerve fibers causes the usual release of acetylcholine. The effect of eserine was to aid the diffusion of acetylcholine from the site of its release in the nerve endings of voluntary muscle into the blood vessels. The experiments of Dale and his colleagues demonstrated quite definitely that the appearance of acetylcholine in the perfusion fluid is not directly or indirectly produced by the contraction of the muscle fibers.

The absence of nuclear division in denervated muscle confirms the findings of Willard and Grau.<sup>54</sup> The hypertrophy of the nucleus and the peripheral accumulation of granules confirm the findings of Tower.<sup>47</sup> Our observations confirm the statement of Hines and Knowlton<sup>55</sup> that the rat is a very satisfactory animal upon which to study the changes in skeletal muscle after denervation. They did not, however, study those changes in the neuromuscular apparatus, as reported in our present paper, because of the obliterating effects of the histologic methods employed by them.

Feiss and Cramer,<sup>56</sup> in discussing the nature of wallerian degeneration, stated that the proliferation of the neurilemmal nuclei and the fragmentation of the myelin sheath must be considered separately. The cytologic changes in the degenerating medullated nerves as correlated with the changes in the axon and myelin sheath are problems for future investigation. The changes revealed by the gold technic, however, point to the fact that the traumatic sectioning and resulting continuous overstimulation of the nerve produce an abnormal breakdown and discharge of neurogenic substances that proceed at a more rapid rate than the process of elaboration. The function of the cell body is required for the synthesis of the normal transmitter in the intact nerve fibers. That acetylcholine is formed in cholinergic nerve fibers was established by Loewi and Hellauer,<sup>57</sup> MacIntosh,<sup>58</sup> and others.

Boeke<sup>59</sup> stated that from a cytologic point of view the nerve endings are in many aspects one of the most interesting parts of the nervous system, and formulated several questions and statements (page 243, *l.c.*) which are a challenge to the anatomists in the study of the neuro-



muscular apparatus. The morphologic evidence on the changes in the denervated neuromuscular apparatus presented in this paper supports the prophetic challenge of Boeke and furthermore substantiates the theory of chemical transmission of nerve impulses advanced by Loewi, Dale, and others. The motor end-plates in living muscle appear to be biologic jet-pumps that discharge either normal or abnormal neurogenic substances into the myoplasm of the muscle fibers.

### SUMMARY

1. The right gastrocnemius muscles of 100 white rats (*Mus norvegicus*) were denervated by sectioning the vagus nerve at the hip. The left legs remained intact and were used as controls. The successful demonstration of the nature of the union of nerve endings and voluntary muscle depended upon the use of the gold-and-teasing method of whole muscle fibers and nerves, checked against results obtained by other histologic technics. Gold had an affinity for acetylcholine and certain degeneration products of the axon and myelin.

2. The dark muscle fibers in normal voluntary muscle, because of their strong affinity for gold, are designated as hyperchrysophilous muscle fibers. They are associated with the periodic discharge of acetylcholine from the motor end-plates and with the diffusion of this chemical transmitter in the myoplasm at the end of relaxation or the onset of contraction.

3. The light voluntary muscle fibers have a weak affinity for gold and are designated as hypochrysophilous muscle fibers. They are associated with the periodic hydrolysis of acetylcholine by cholinesterase during the period of full, active contraction.

4. After denervation there is a progressive loss of the dark hyperchrysophilous muscle fibers and motor end-plates, which involved 60 per cent of them 72 hours after denervation. This coincides with the beginning of fibrillation and with the progressive disappearance of acetylcholine. At about the 10th to 15th day after denervation dark muscle fibers are totally absent. The atrophic muscle fibers become achrysophilous. The rates of these pathologic changes vary among animals of the same species as well as among different species.

5. There is a progressive and centrifugal transport of degenerative materials from the distal segment of the sectioned nerve to the denervated muscle. This produces large pleomorphic neurosomes in the denervated myoplasm. This phenomenon is accelerated by the use of d-tubocurarine chloride, particularly when followed by electrical stimulation of the distal stump of the sciatic nerve. There is a substantial

transfer from nerve to muscle. Giant muscle fibers are produced occasionally, composed largely of neurogenic material. The giant fibers of primary muscular atrophy and dystrophy need reinvestigation.

6. The bloated subsarcolemmal nuclei of the denervated muscle become surrounded by an increased amount of granular material coincident with the persistent activity of cholinesterase, as found by Nachmansohn and John,<sup>7</sup> 3 days after nerve section when conductivity had disappeared in the axon.

7. The hyperchrysophilous neurosomes may be defined as ephemeral pleomorphic bodies discharged into the myoplasm of striped muscle from superpermeable terminal axons of motor end-plates. These neurosomes are composed of very fine granules, droplets, vacuoles, and elongated streamers that vary in size and staining capacity with gold, silver, and lipoidal stains.

8. Motor end-plates in living muscle appear to be biologic jet-pumps that discharge neurogenic substances into the myoplasm of the muscle fibers. Voluntary muscle has a dual composition of neurogenic and myogenic substances.

9. Muscle atrophy appears to depend upon a decrease or total inhibition of the neurogenic discharge into muscle, and hypertrophy upon an increase of this neurogenic discharge. The changes in the blood supply appear to be secondary phenomena.

10. Anatomic evidence of progressive degeneration, as contrasted with simultaneous degeneration, occurs in the distal stump of the sciatic nerve. This process may be accelerated by experimental means with resulting massive discharge of degenerated nervous material into the denervated muscle.

11. The union of nerve and muscle appears to be one of periodic anatomic continuity which results in the confluence and compounding of the neurogenic and myogenic substances. Gold chloride, after initial acidulation of the living muscle, appears to possess a selective affinity for the normal and abnormal neurogenic substances.

12. The experimental anatomic evidence appears to support the claim that there are morphologic as well as chemical transmitters of normal and abnormal nerve impulses demonstrable by selective histologic methods.

Grateful acknowledgment is expressed for technical assistance to: Estelle Downer, Frances Toomey, Eli Socolof, and to the 1944-1945 freshman classes for their combined contribution of over 30,000 man-hours in teasing muscle in this and related problems; to Dr. Guy Kasten Tallmadge for his valuable suggestions in the reading of the manuscript; and to Drs. C. F. Church and H. S. Newcomer of E. R. Squibb and Sons for the d-tubocurarine used in experiments.

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[ Illustrations follow ]

The photomicrographs of Figures 1 to 82 are from teased whole muscle fibers (gastrocnemius muscle) and motor end-plates of the white rat (*Mus norvegicus*), previously prepared by the gold technic. The photographs were direct contact prints from negatives exposed through the microscope and not subsequently enlarged. They may, therefore, be compared with those of the white rat and the chameleon previously published.<sup>1,2</sup> In the plates, "epa" designates the epilemmal axon; "hya," the hypolemmal axon; "Ns," neurosomes or neurogenic particles discharged from motor end-plate jet-pumps into the myoplasm either as fine, nebulous sprays, large agglutinated clusters, or periodic series of globules; "Kg," extra-axonic Kühne's granules; "gmf," giant muscle fiber; "b.v.," blood vessel; "nuc.," nucleus; "gran.," granules. There has been no retouching of negatives or prints. The time after section of the nerve is designated in each legend.

#### PLATE 241

FIG. 1. Spray of medullated nerve fibers and terminal motor end-plates from a relatively normal gastrocnemius muscle of a rat killed by ether. There is pleomorphism and variation in size and impregnation capacity for gold of the motor end-plates and muscle fibers. The narrow, dark muscle fibers may be the initial phase of a nebulous and periodic diffusion of granular acetylcholine at the onset of contraction. Choline and acetylcholine react with gold chloride *in vitro* to produce either choline aurichloride or acetylcholine aurichloride. The wide, light muscle fibers may represent the phase of contraction after the diffused acetylcholine has been destroyed by the cholinesterase. This normal variation of muscle fiber types may be the morphologic expression of the fractional contraction in a muscle, and the differential diffusion alternating with the destruction of acetylcholine in the different fibers of the same muscle. The motor end-plates in the dark muscle fibers are retracted and surrounded by the granules of Kühne. In the light muscle fibers the motor end-plates are usually expanded and there is a decrease in the amount of the granules of Kühne.  $\times 250$ .

FIG. 2. Spray of medullated nerve fibers depleted of motor end-plates in the gastrocnemius muscle 72 hours after the degenerative cut of the sciatic nerve. The motor end-plates are replaced by fine granules surrounding light, oval spaces occupied by the nuclei of the sole. The epilemmal axons are increased in diameter, and in some terminals there are swollen cysts. The absence of the dark type of muscle fibers in this microscopic field may possibly be related to the failure of normal transmission of acetylcholine granules or the failure of the normal nebulous diffusion of the granular transmitter substance after discharge from the end-plates into the myoplasm.  $\times 250$ .

FIG. 3. Spray of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 120 hours after the degenerative cut of the sciatic nerve. The muscle was curarized by the intraperitoneal injection of d-tubocurarine chloride and the distal segment was then stimulated for 30 seconds at the rate of 5 per second with no muscular response. There is depletion of the material in the epilemmal axons and an enormous accumulation of this neurogenic material in the end-plate with periodic discharges into the muscle fiber. The exhaustion from the axonic spray of its specific substance with an affinity for gold and the accumulation of this substance at the end-plate and in the muscle constitute conclusive evidence of the substantial transfer of some substance from nerve to muscle.  $\times 250$ .

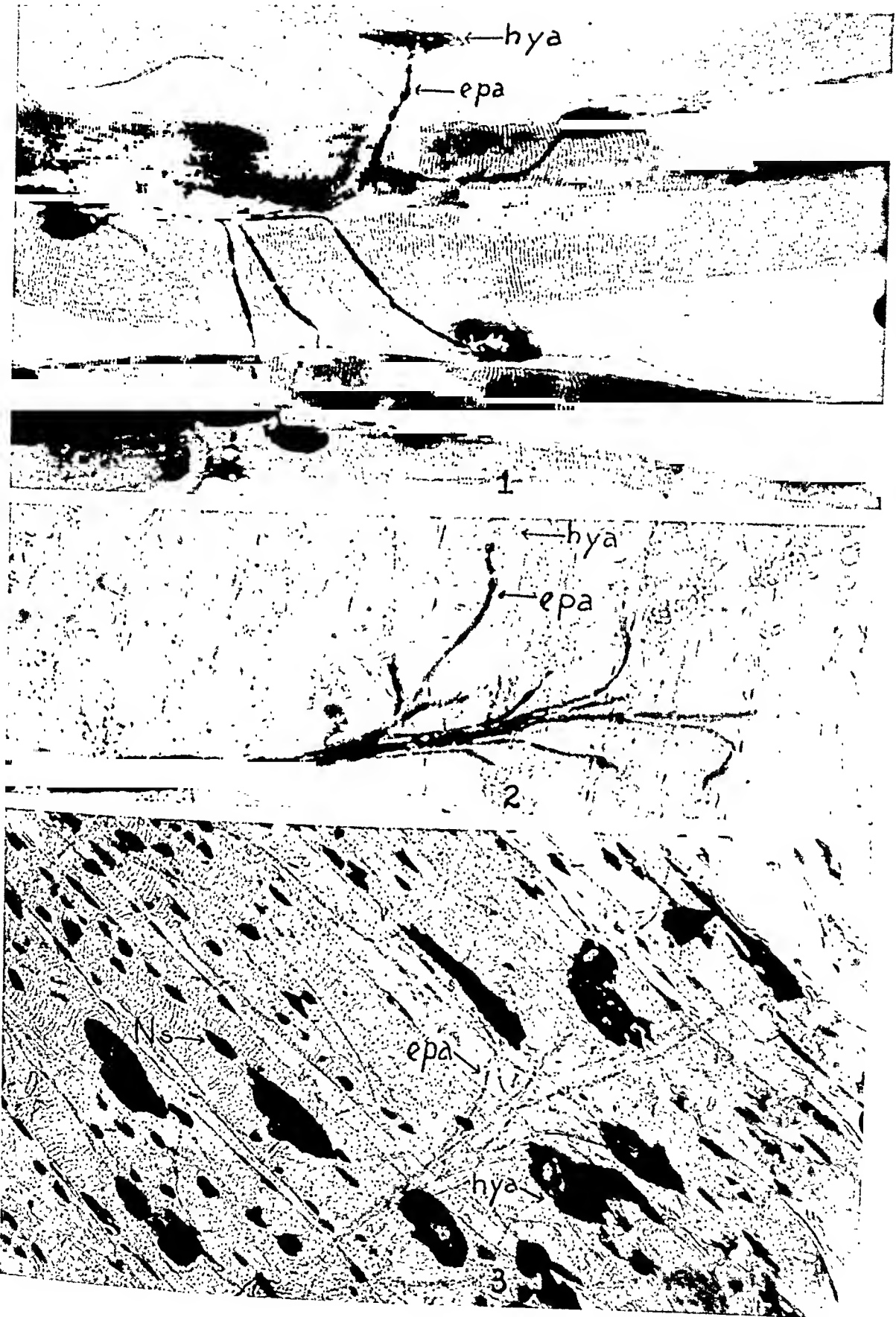




PLATE 242

FIGS. 4 and 5. Sprays of medullated nerve fibers and motor end-plates 24 hours after section of the sciatic nerve. There is a beginning loss of the dark type of muscle fiber. The epilemmal axons are definitely beaded (Fig. 4). In some places the end-plates have a diminished affinity for the gold. In other places in the same muscle all of the end-plates are retracted and have an increased affinity for gold (Fig. 5). Some hypolemmal axons have round or oval cystic enlargements. This is a histologic sign of an abnormal and uniform stimulation of the motor units of nerves and a beginning loss of the fractional contraction of the fibers in the same muscle. In other locations of the same muscle all of the end-plates in the field may be abnormally expanded. The abnormal contraction or expansion of all of the motor end-plates in a field is evidence of uniform overstimulation and overexcitation of the motor units of the muscle and loss of the fractional contraction of the muscle fibers normally controlled by the intact nervous system.  $\times 200$ .

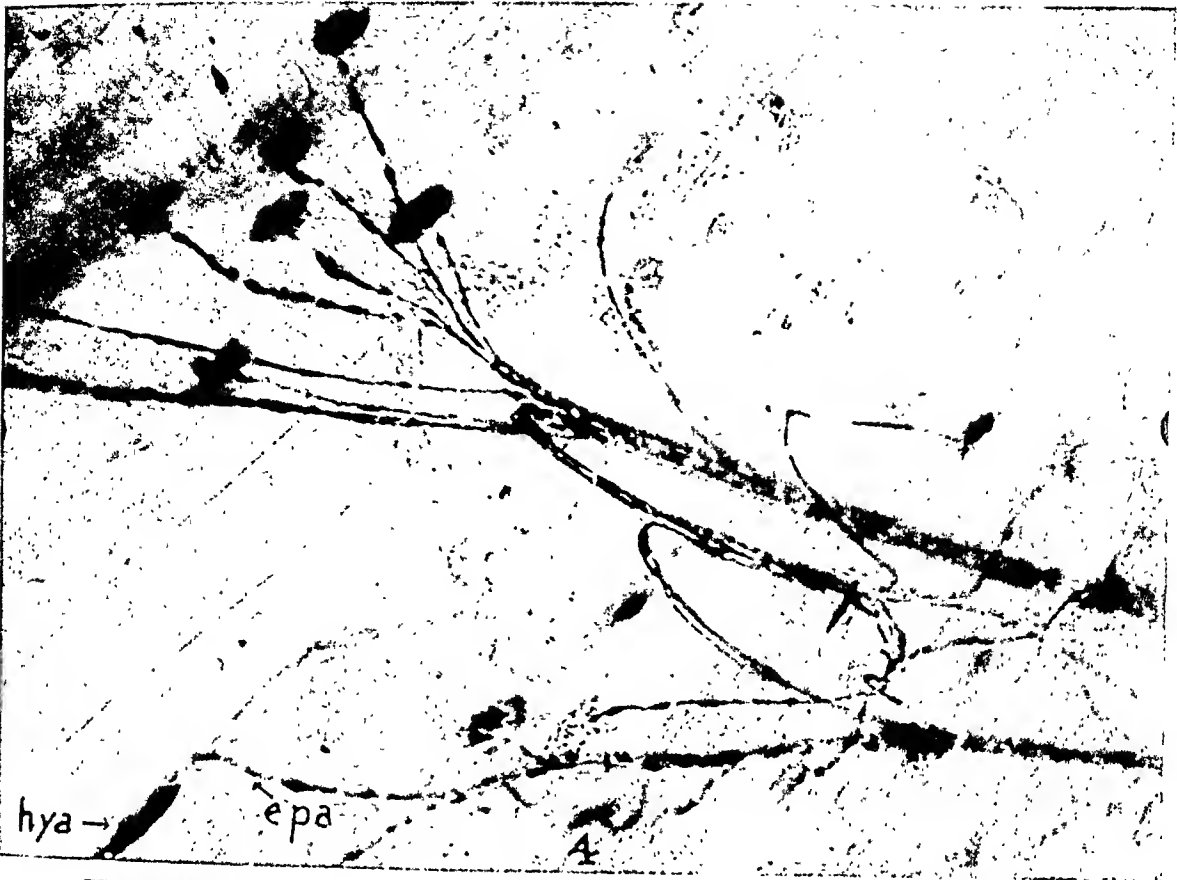


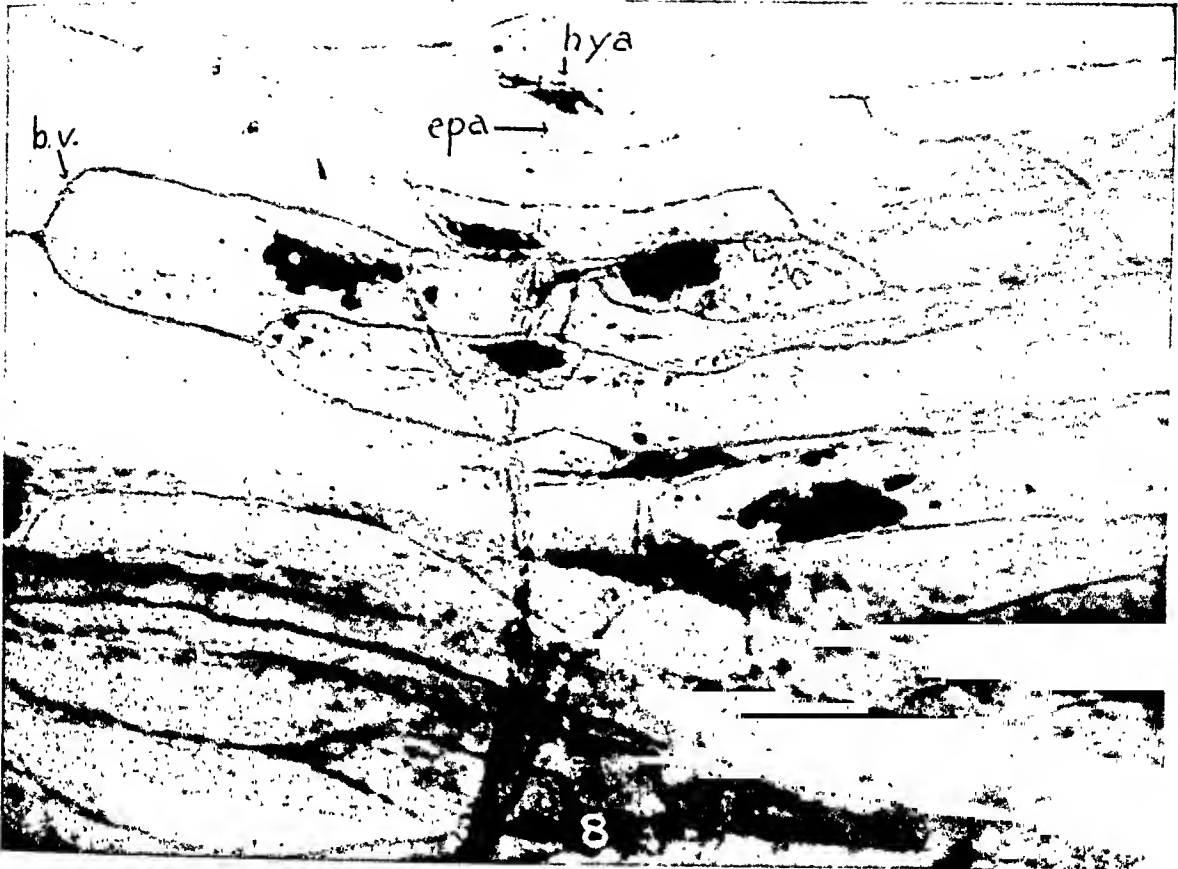
PLATE 243

FIGS. 6 and 7. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 48 hours after the degenerative cut. There is a progressive loss of visible structural organization in muscle fibers of the dark type. In many places all of the motor end-plates in a specific field may be either retracted with increased affinity for gold (Fig. 6) or expanded with a decreased affinity for gold (Fig. 7). This is a histologic sign of an abnormal and uniform overstimulation of the motor units and a loss of the fractional contraction of the muscle fibers. There is a dark granular or flaky appearance of the muscle fiber, which is a histologic sign of the beginning loss of the normal nebulous diffusion of the granular transmitter substance discharged from the motor end-plate into the myoplasm of the muscle fiber.  $\times 250$ .



PLATE 244

FIGS. 8 and 9. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 48 hours after the degenerative cut. There has been an advance in the loss of structure of the muscle fibers. Some of the hypolemmal axons are replaced by fine granules. In some places there is a dark, coarse, granular to flaky appearance of the muscle fibers which is a histologic sign of the beginning loss of the normal periodic diffusion of the fine granular transmitter substance. In some nerve trees the epilemmal axons are fragmented and have a decreased affinity for gold (Fig. 8), while in other places there is an increased capacity to take the gold with little evidence of fragmentation (Fig. 9). Since the technic was standardized by running the normal and abnormal muscles simultaneously through the same fluids for the same periods of time, this is considered a histologic sign of a periodic decrease alternating with an increase in the quantity of material in the epilemmal axons. There is a definite hyperemia of the intramuscular blood vessels and capillaries, demonstrated by the increased diameter of the lumen and the packing of these vessels with red blood cells. In other locations the capillaries are collapsed and devoid of cellular elements. This is comparable to the conditions produced by experimental traumatic and thermal shock and by the local injection of lactic and other acids into the muscle.  $\times 250$ .



## PLATE 245

FIG. 10. Spray of medullated nerve fibers and terminal motor end-plates from a relatively normal gastrocnemius muscle of a rat killed by ether. There is pleomorphism and variation in the size and impregnation capacity for gold of the motor end-plates and muscle fibers. The narrow dark muscle fiber associated with a dark retracted end-plate may represent the evanescent phase of the nebulous diffusion of the fine granular acetylcholine at the onset of the contraction of the muscle fiber. The wide light muscle fibers frequently associated with expanded end-plates with a decreased affinity for gold may represent the phase of active contraction of the muscle fiber after the diffused acetylcholine has been hydrolyzed by cholinesterase. This alternation of dark and light auriphilic fiber types may be the morphologic expression of fractional contraction in a normal muscle, and of normal periodic diffusion, alternating with destruction, of the fine granules of acetylcholine in different fibers of the same muscle. The differential capacity to take gold may be a morphologic measure of the quantity of neurogenic substances diffused into the myoplasm of the muscle fiber. The normal and abnormal muscles were concurrently subjected to the same standardized technic.  $\times 250$ .

FIG. 11. Spray of medullated nerve fibers and granular motor end-plates in the gastrocnemius muscle 72 hours after section of the sciatic nerve. The motor end-plates are replaced by fine granules surrounding the light oval spaces occupied by the nuclei of the sole. The epilemmal axons in this field have a decreased affinity for gold in comparison to the relatively normal axons (Fig. 10). There is a dark, coarse, granular or flaky appearance of the muscle fibers, which is a histologic sign of beginning loss of normal diffusion of the fine granules of the transmitter substance discharged from the motor end-plate into the myoplasm of the muscle fiber. There is likewise a definite loss of structure in the dark type of muscle fiber. This may well represent the loss of the normal initial phase of periodic diffusion of the normal neurogenic transmitter substance associated with the production of dark muscle fibers. These dark muscle fibers progressively disappear after the degenerative cut of the nerve, and their disappearance parallels the loss of transmission of acetylcholine from the motor nerve endings into muscle.  $\times 250$ .





## PLATE 246

FIG. 12. Spray of medullated nerve fibers and terminal granular degeneration of the motor end-plates in the gastrocnemius muscle 5 days after the degenerative cut of the sciatic nerve. These motor end-plates are replaced by fine granules surrounding the light oval spaces occupied by the nuclei of the sole. There is a loss of structure in the dark type of muscle fibers. The fibers are becoming narrower than normal and the sarcolemmal nuclei are more evident in some places than in normal muscle with the gold technic. In some places there is definite fragmentation of the epilemmal axons and in others a depletion of the axonic material.  $\times 250$ .

FIG. 13. Spray of medullated nerve fibers and terminal granular degeneration of the motor end-plates in the gastrocnemius muscle 7 days after the degenerative cut of the sciatic nerve. These motor end-plates are replaced by fine granules. The nuclei of the sole plate are beginning to take the gold stain in certain places. There is a loss of structure of muscle fibers of the dark type. The fibers are becoming narrower than normal and the visibility of the sarcolemmal nuclei in some places is increased beyond the normal with gold. In certain places there is definite fragmentation of the epilemmal axons, and in others a depletion of the axonic material. The axonic material in the nerve trunk is fragmented into rounded droplets, elongated bodies with rounded ends, and fusiform structures. In following a nerve trunk throughout its course, first without and then within a muscle, this fragmented axonic material appears to travel in a periodic manner in a centrifugal direction. This material is then discharged from the distal nerve terminal into the muscle.  $\times 250$ .

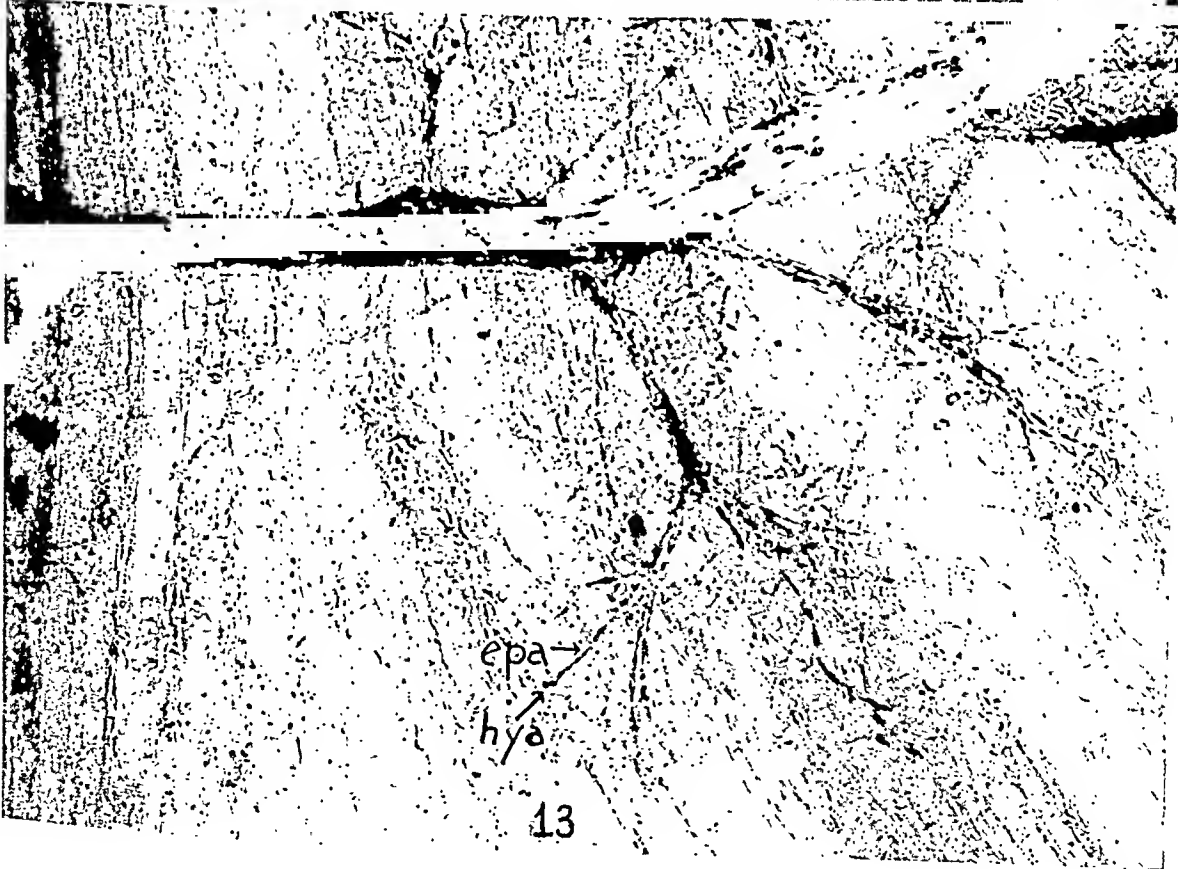


PLATE 247

FIGS. 14 and 15. Sprays of medullated nerve fibers and terminal granular degeneration of the motor end-plates in the gastrocnemius muscle 9 days after the degenerative cut of the sciatic nerve. These motor end-plates are replaced by fine granules surrounding the light oval spaces occupied by the nuclei of the sole. There is a definite loss of the dark type of muscle fiber. In these sprays the epilemmal axons are loaded with material that has a strong affinity for gold. There is very little evidence of fragmentation of the epilemmal axons. After the degenerative cut of the nerve the fragmentation of the epilemmal axon appears and disappears with the progressive and intermittent advance in a peripheral direction of the degenerated axonic material and its discharge centrifugally in the region of the degenerated motor end-plates. There is no evidence of hypolemmal axons. These have been completely replaced by rounded and oval islands of granules. Some of the epilemmal axons have dichotomous divisions. In other places the epilemmal axon is single and terminates in a rounded or frayed end which gradually disappears by confluence with the myoplasm of the muscle fiber. Figure 14,  $\times 400$ ; Figure 15,  $\times 850$ .

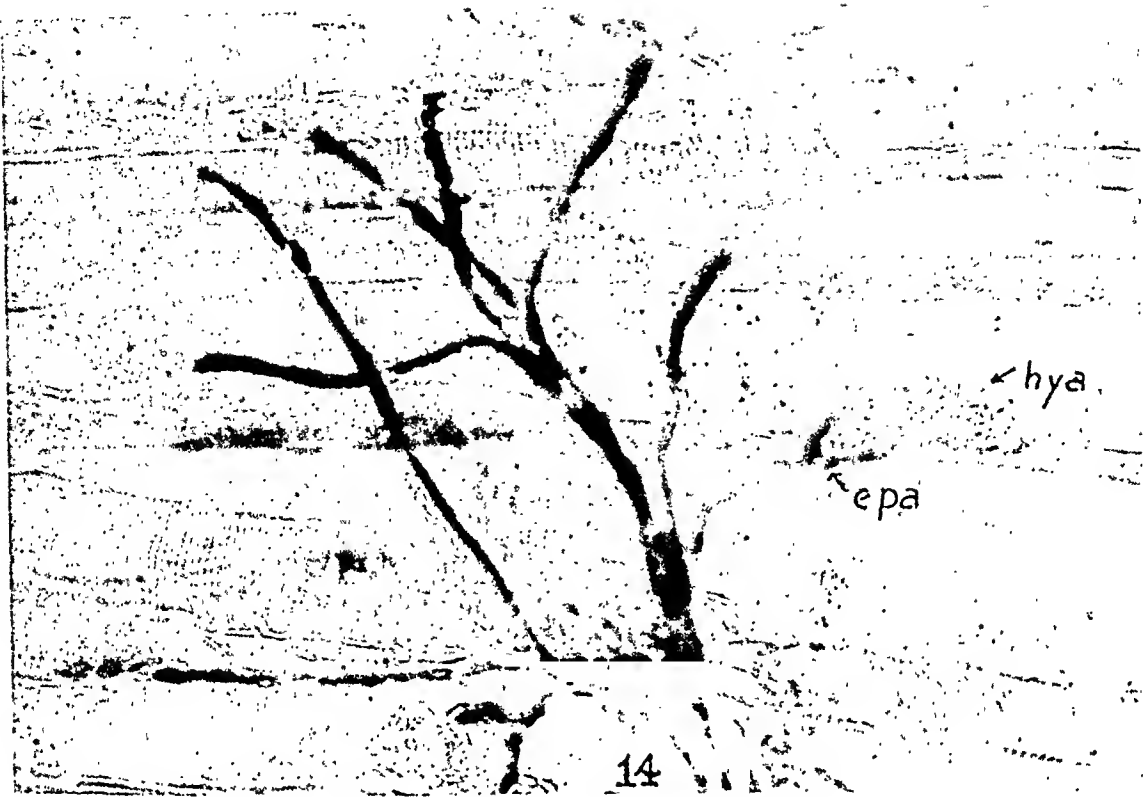


PLATE 248

FIGS. 16 and 17. Sprays of medullated nerve fibers undergoing depletion of their axonic material in a centrifugal direction in a gastrocnemius muscle 10 days (Fig. 16) and 12 days (Fig. 17) respectively after the degenerative cut of the sciatic nerve. The discharged axonic material that has a strong affinity for gold is accumulating in pleomorphic masses within and around the region of the degenerated motor end-plates. The faint, ghost-like outline of the epilemmal axons depleted of substances that have a strong affinity for gold is in striking contrast to that of the accumulated material, with the capacity to take the gold, discharged in a centrifugal direction into the region of the degenerated motor end-plates.  $\times 250$ .

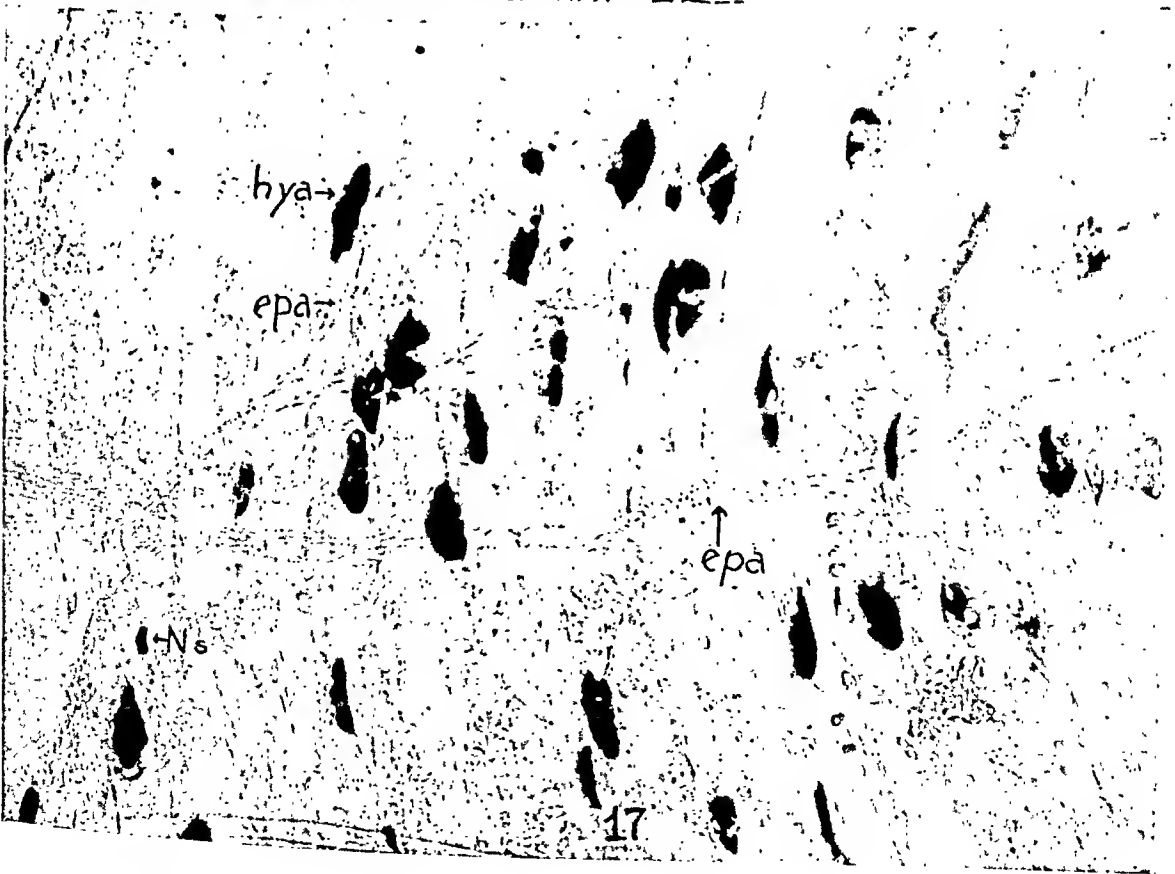


PLATE 249

FIGS. 18 and 19. Sprays of medullated nerve fibers depleted of their axonic auriphilic material which is discharged in a centrifugal direction into the gastrocnemius muscle 14 days (Fig. 18) and 20 days (Fig. 19) respectively after the degenerative cut of the sciatic nerve. The discharged axonic material with a strong capacity to combine with gold is accumulated in pleomorphic masses within and around the region of the degenerated motor end-plates. Some of these pathologic masses or neurosomes are found scattered in the myoplasm of the muscle fiber. The faint, ghost-like outline of the epilemmal axons depleted of their substance that has an affinity for gold is again in striking contrast to the accumulated gold-impregnated material discharged in a centrifugal direction. The muscle fibers 20 days after the degenerative cut have lost the appearance of the normally functioning dark type, and they are considerably narrower in comparison with the normal. The anatomic evidence is incontrovertible that the histologic dark type of muscle fiber is related to a normally functioning nerve supply. This functional dark type of muscle fiber appears to be produced by the normal diffusion into the myoplasm of the fine granules of acetylcholine discharged from the motor end-plate. (For comparison with Figs. 1, 10, and 22).  $\times 250$ .

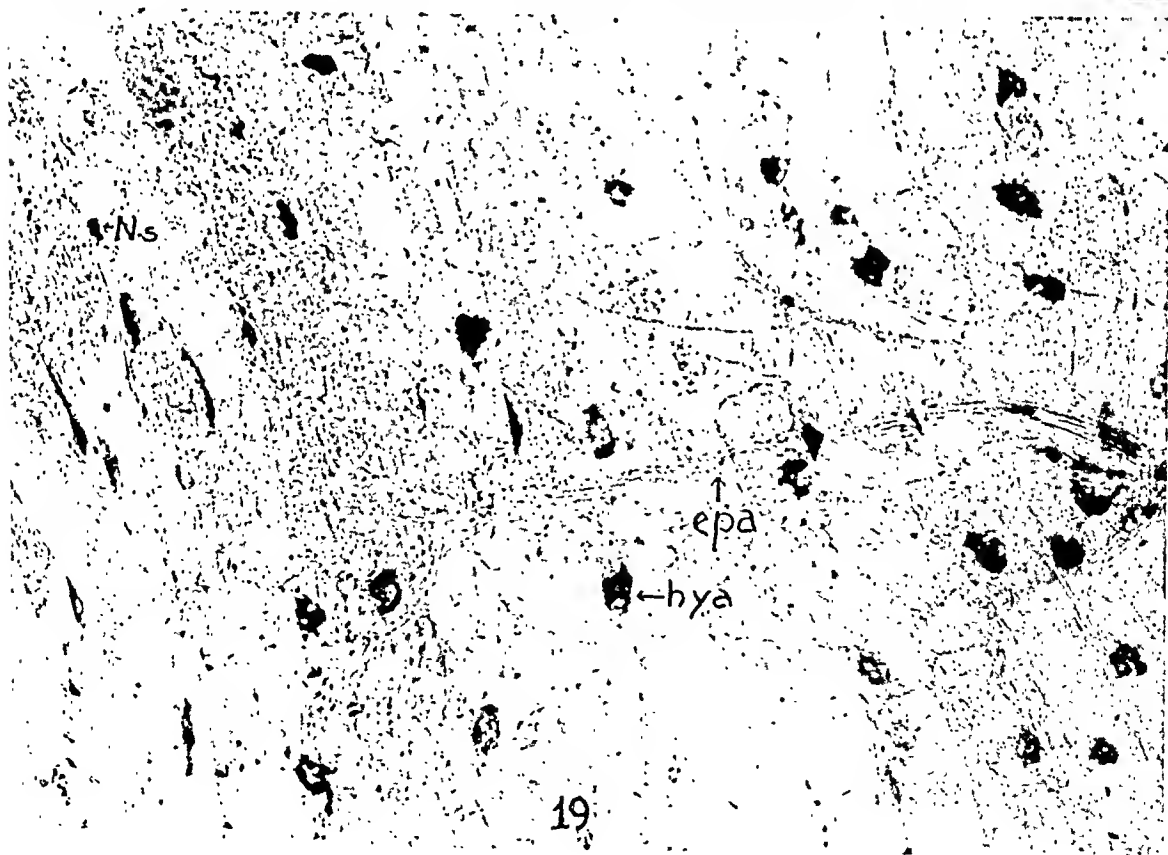
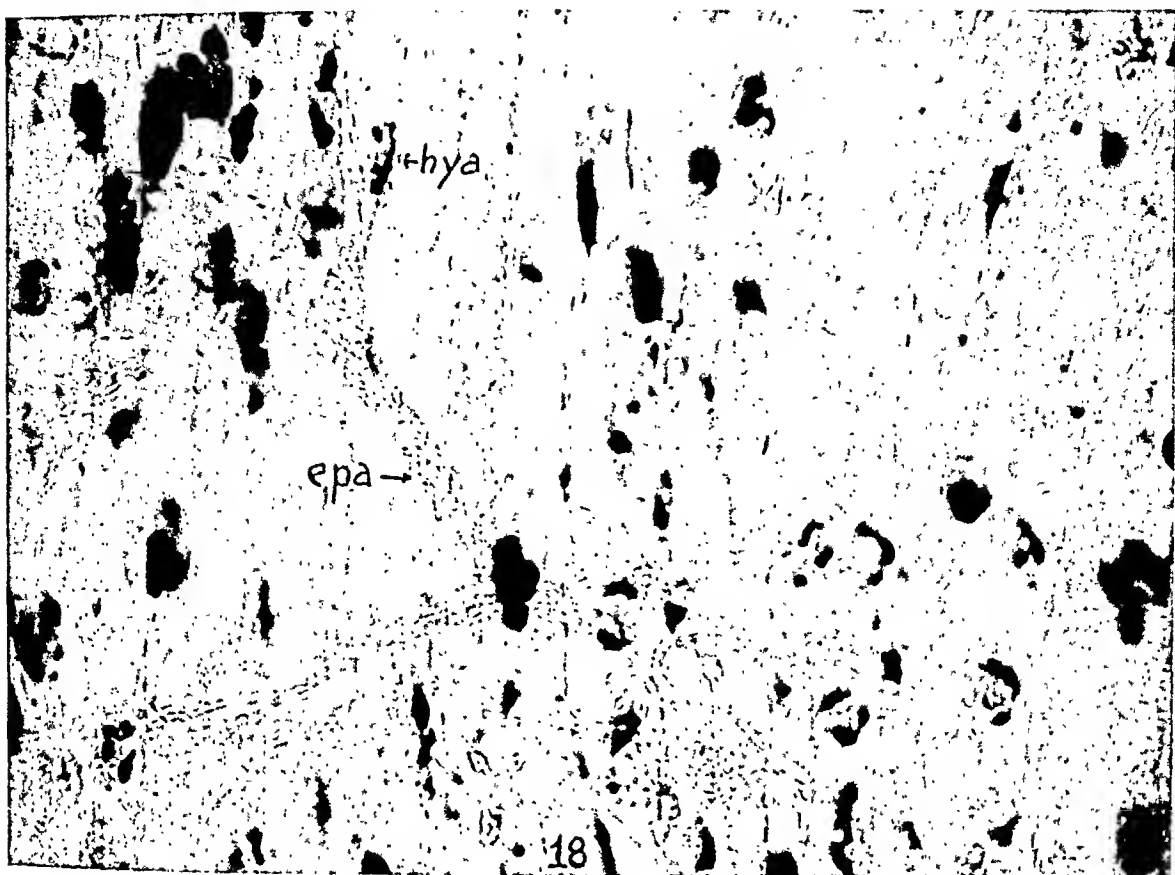
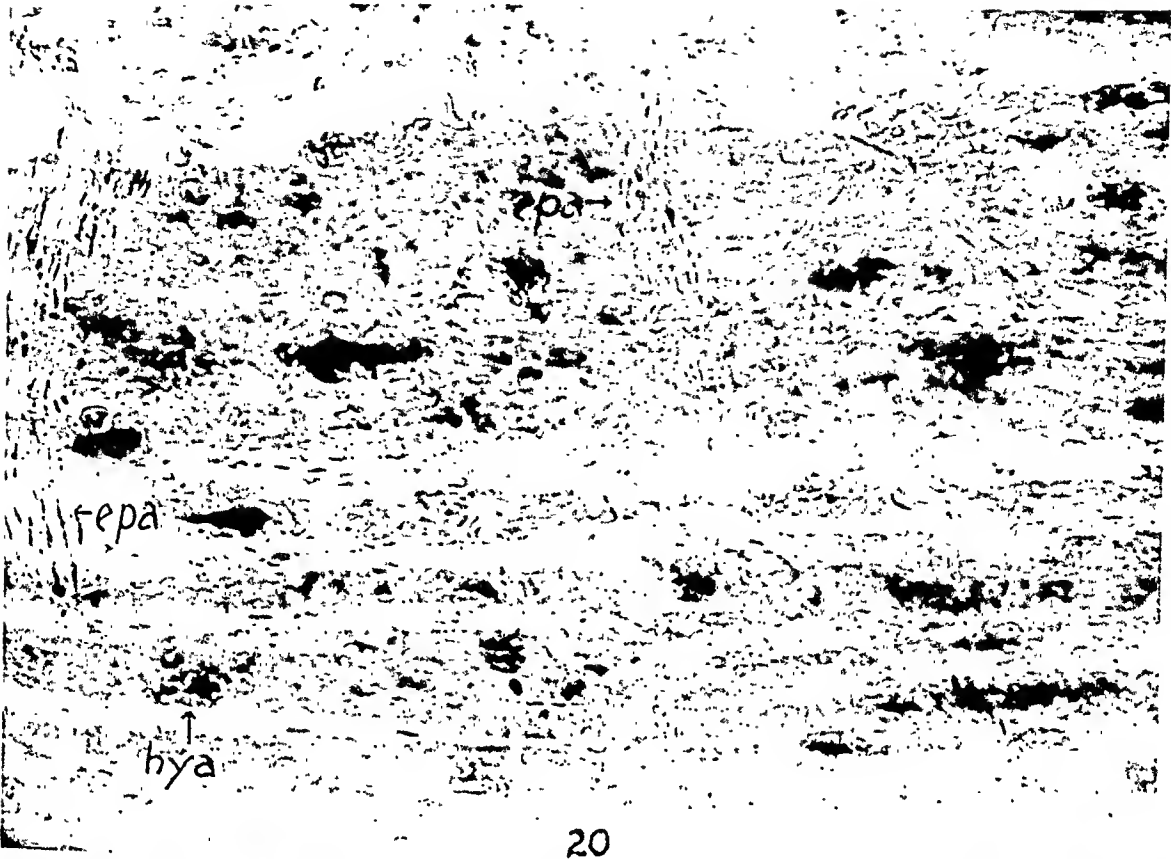




PLATE 250

FIGS. 20 and 21. Sprays of medullated nerve fibers depleted of their axonic material in the gastrocnemius muscle 30 days after the degenerative cut of the sciatic nerve. The axonic material, which was discharged in a centrifugal direction, is seen only in relation to a few of the degenerated end-plates. The locations of the degenerated end-plates are indicated principally by clusters of sole-plate nuclei which are surrounded by the granular material secreted by these nuclei. This granular material has an affinity for gold slightly less than that of the material discharged by the hypolemmal axons. The clusters of nuclei are detected as clear, rounded, or oval spaces surrounded by dark granules (Fig. 20). In some places, in the same muscle, the myoplasm is aggregated into broad or narrow, dark, transverse bands alternating with light ones (Fig. 21). In the dark bands the cross striations are either exceedingly fine or they are completely absent because of the opacity of the dense aggregation of the myoplasm. The light bands are occupied by cross striations which are farther apart than those in the dark bands. This dark and light transverse banding of the narrow muscle fibers is produced by the slow, irregular fibrillation of the muscle fibers. This incoordinate, ceaseless, slow, vermiform fibrillation of the denervated muscle fibers begins about 72 hours after the degenerative cut of the sciatic nerve, and is correlated with loss of transmission of acetylcholine, which, normally, chemically tunes the muscle fiber to the higher pitch or frequency of fast muscle contractions and maintains its capacity to respond adequately to high-frequency stimulation. This chemical tuning process is roughly analogous to the mechanical tuning of the strings of a piano or violin for normal response at a higher frequency than is possible in strings sagging between their supports.  $\times 250$ .



# PLATE 251

FIG. 22. Spray of medullated nerve fibers and terminal motor end-plates from a relatively normal gastrocnemius muscle of the rat killed by ether. There is definite pleomorphism with variation in the size, shape, and impregnation capacity for gold of the motor end-plates and muscle fibers. The narrow, dark muscle fibers may represent the initial phase of the fine, nebulous diffusion of granular acetylcholine at the beginning of contraction of certain muscle fibers. The wide, light muscle fibers may represent the phase of active contraction of some muscle fibers after the diffused acetylcholine has been destroyed by hydrolysis through the action of the cholinesterase. This variation and alteration of fiber types in the same muscle fiber may be the morphologic expression of the fractional contraction of the fibers in a muscle and the active periodic diffusion, alternating with the destruction by hydrolysis, of acetylcholine in the different muscle fibers of the same muscle related to a normal innervation. The same muscle fiber at different periods of time may be either dark or light by gold impregnation, dependent upon the phase of active diffusion or hydrolytic destruction respectively of acetylcholine granules. The dark and light muscle fibers, according to this concept, are morphologic variations that parallel the different periods of functional activity. The dark muscle fiber loses that character when the normal secretion of acetylcholine is abolished by denervation.  $\times 150$ .

FIG. 23. Spray of medullated nerve fibers depleted of their axonic and degenerated myelin materials in the gastrocnemius muscle 10 days after the degenerative cut of the sciatic nerve. There is a definite loss of structure of the different types of muscle fibers. The dark type of muscle fiber has been eliminated. The epilemmal axons are definitely depleted of the fragmented axonic and myelinic materials. This depletion of the epilemmal axons of their gold-impregnated materials is due to the centrifugal discharge of the degenerated axonic substance into and around the degenerated motor end-plates. There is, likewise, a discharge of neurosomes throughout the muscle fibers. This morphologic change was produced by the intraperitoneal injection of d-tubocurarine chloride followed by electrical stimulation of the degenerating peripheral stump of the cut sciatic nerve. The demonstration of the pathologic structure of the normal or abnormal transmitter substance may be demonstrated best experimentally by accelerating the discharge of the axonic material into the muscle and by preventing or delaying its normal diffusion into the myoplasm of the muscle fiber.  $\times 150$ .

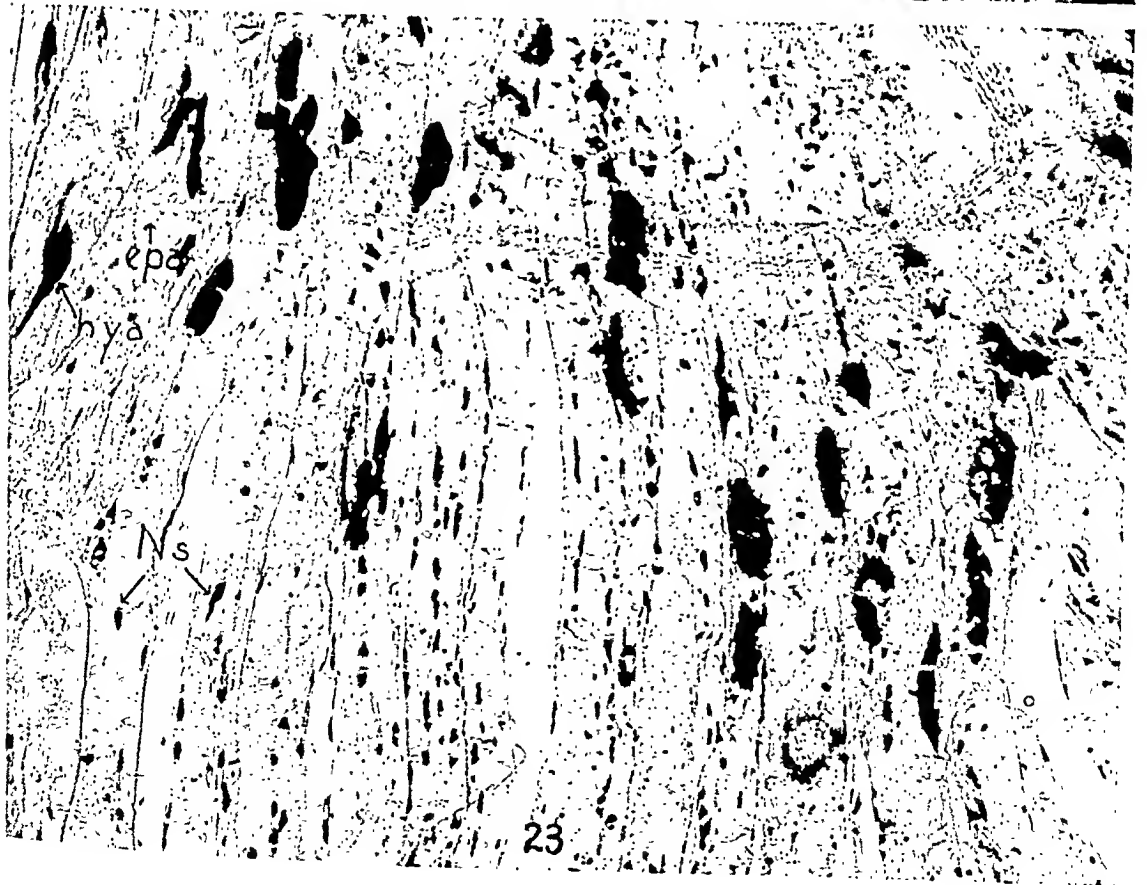
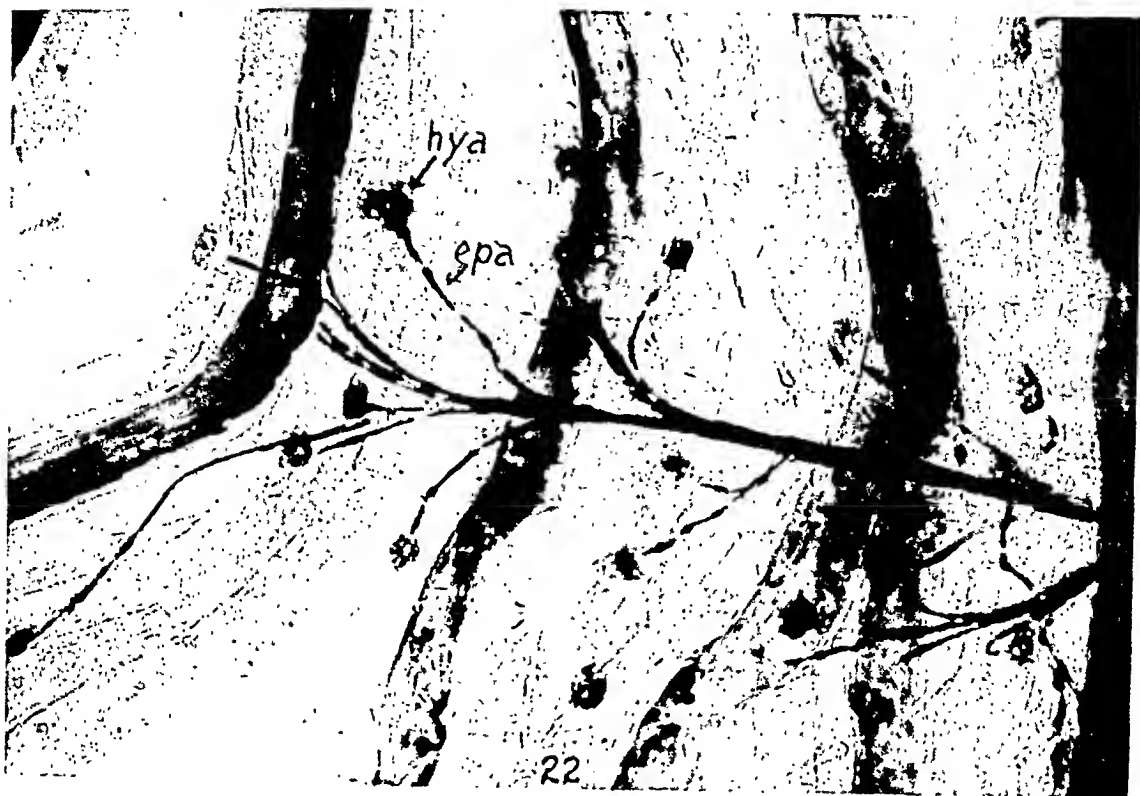


PLATE 252

FIGS. 24 and 25. Sprays of medullated nerve fibers depleted of their axonic and myelin material with the degenerated motor end-plates surrounded by augmented material with a strong affinity for gold, from the gastrocnemius muscles of rats, 12 (Fig. 24) and 14 (Fig. 25) days, respectively, after section of the sciatic nerves. The intraperitoneal injection of d-tubocurarine chloride was followed by electrical stimulation of the distal segment of the cut nerve for 30 seconds at the rate of 5 per second. There is practically complete depletion of the epilemmal axons of the substances with an affinity for gold. This gold-impregnated material has been discharged in a centrifugal direction into and around the degenerated motor end-plates. This discharge has been projected in a very profuse manner throughout the muscle fibers. There is a definite loss of the dark type of muscle fiber as a result of denervation (for comparison with Fig. 22, normal innervation of gastrocnemius muscle). The pathologic structure of this abnormal transmitter substance is due to the experimental acceleration of the discharge and the lack of normal diffusion of this abnormal transmitter substance into the myoplasm of the muscle fiber. The irregular discharge of the nondiffusible nervous substances into the muscle apparently prevents the appearance of the functional dark type of muscle fiber, which appears to be the product of the discharge and uniform nebulous diffusion in a periodic manner of fine granules from a normal innervation. These fine neurogenic granules may be interstitial granules of Kölliker found in normal muscle. The large neurosomes may represent not only an agglutination of the granules of Kölliker, but also the discharge of abnormal substances into the muscle.  $\times 150$ .

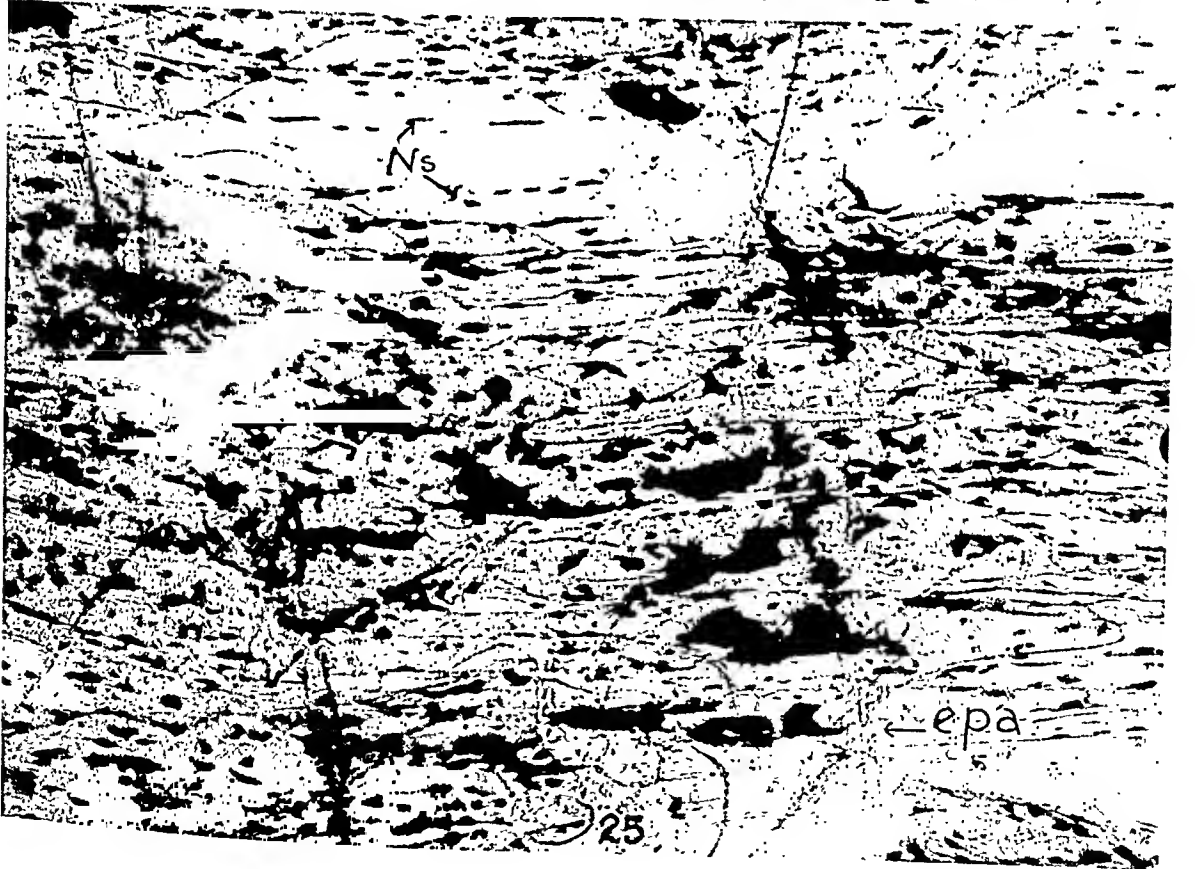
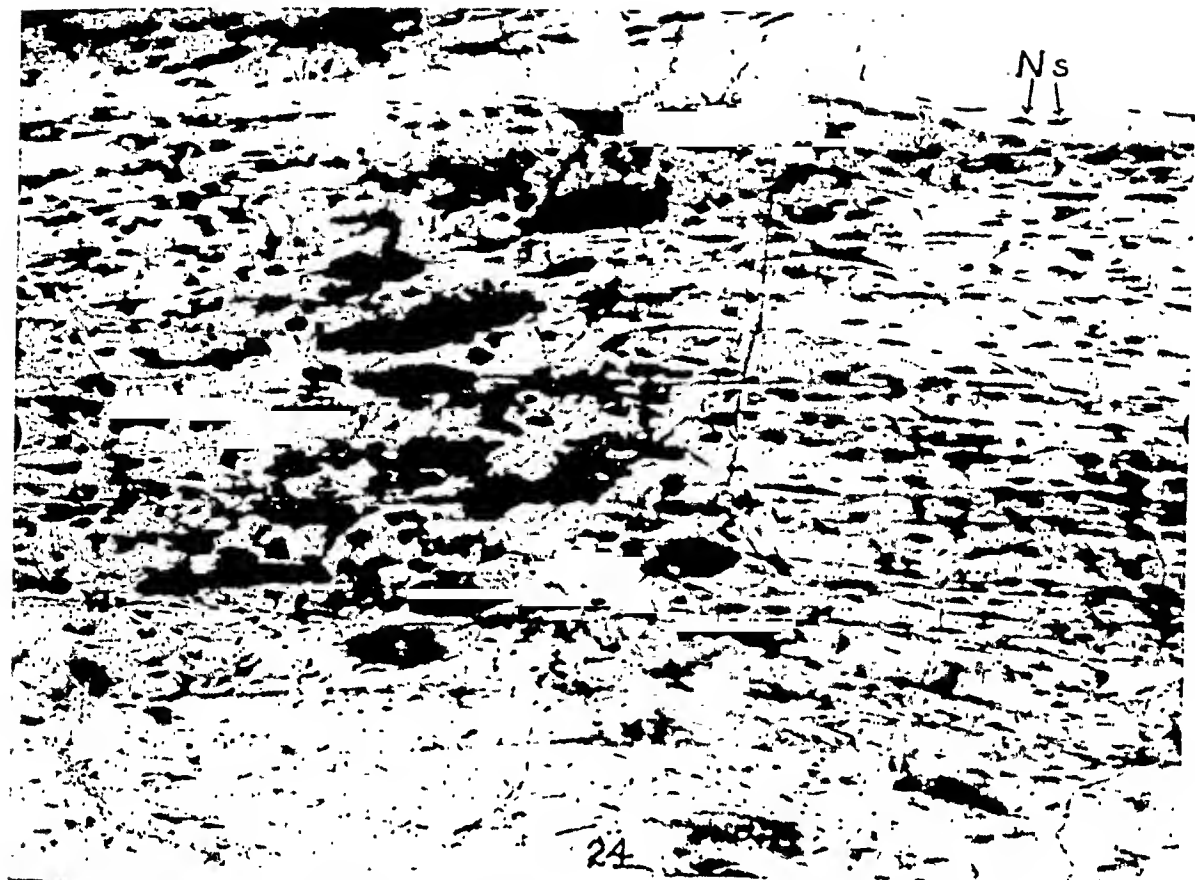


PLATE 253

FIGS. 26 to 29. Sprays of medullated nerve fibers depleted of their axonic and myelin materials, and the degenerated motor end-plates surrounded by an augmented amount of material with a strong affinity for gold in the gastrocnemius muscle of the rat 15 days after the degenerative cut of the sciatic nerve. The intraperitoneal injection of d-tubocurarine chloride was followed by a short electrical stimulation of the distal segment of the cut nerve for a duration of 30 seconds at the rate of 5 per second. There is practically complete depletion from the epilemmal axons of the substances with an affinity for gold. This gold-impregnated material has been discharged in a centrifugal direction into and around the degenerated motor end-plates. This discharge has likewise occurred as multiple series of small, round droplets with a strong affinity for gold. Two to ten little droplets that have a strong affinity for gold are found in each series. The beginning and the end of each series is composed of droplets smaller than those at the middle of the series. Some muscle fibers do not contain these droplets of neurogenic material. This pleomorphism of the material discharged by the end-plate is related to the different phases in the abnormal secretion from the motor nerve terminal into the myoplasm of the muscle fibers.  $\times 150$ .

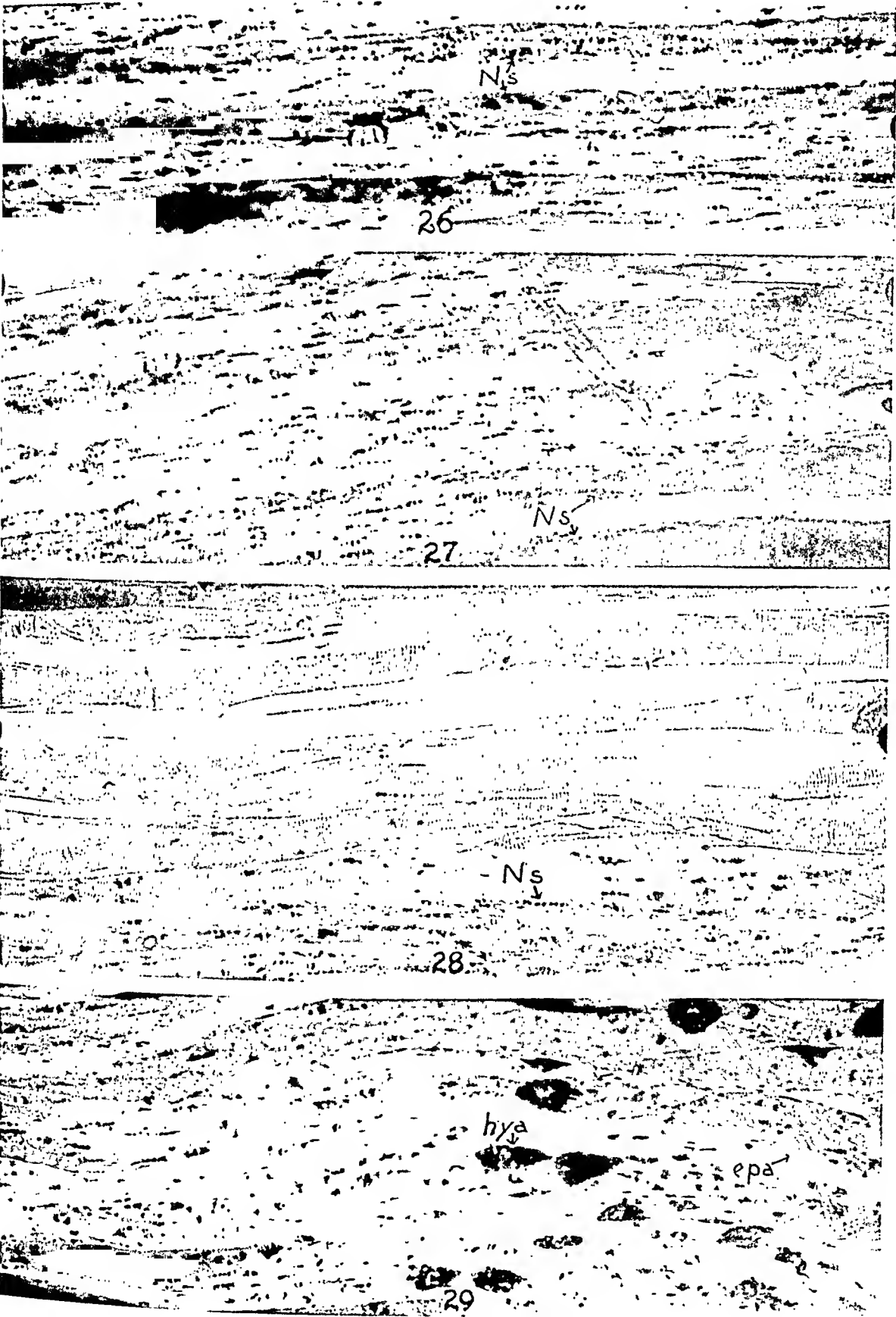




PLATE 254

FIGS. 30 and 31. Sprays of medullated nerve fibers depleted of their degenerated axonic and myelin materials, and degenerated motor end-plates surrounded by an augmented amount of substances with a strong affinity for gold in the gastrocnemius muscles of rats, 5 (Fig. 30) and 10 (Fig. 31) days, respectively, after the degenerative cut of the sciatic nerves. The intraperitoneal injection of d-tubocurarine chloride was followed by a short electrical stimulation of the distal segment of the cut nerve for a duration of 30 seconds at the rate of 5 per second. There is practically complete depletion from the epilemmal axons of the substances with an affinity for gold. This gold-impregnated material has been discharged in a centrifugal direction into and around the degenerated motor end-plates. This discharge has, likewise, occurred in a very profuse manner through the myoplasm. There is a definite loss of the functional dark type of muscle fiber (for comparison with Fig. 22, normal innervation of gastrocnemius muscle). The pathologic structure of this abnormal transmitter substance is due to the experimental acceleration of the abnormal discharge and the lack of the normal nebulous and periodic diffusion of the fine, granular transmitter substance into the myoplasm. The profuse discharge of nondiffusible and abnormal nervous particles into the muscle apparently prevents the appearance of the functional dark type. Three experimental factors are working concurrently to produce the large amount of neurogenic material in muscle: the curare-like effect on muscle of nerve sectioning, the active effect of relatively pure curare upon the myoplasm, and the accelerated discharge of relatively nondiffusible or delayed-diffusible degenerated axonic and myelin materials produced by the electric stimulation of the distal stump of the cut sciatic nerve. With the discharge of great masses of the abnormal axonic material into the muscle there is a concomitant disappearance of this material in both the epilemmal and hypolemmal axons of the motor end-plate.

× 350.

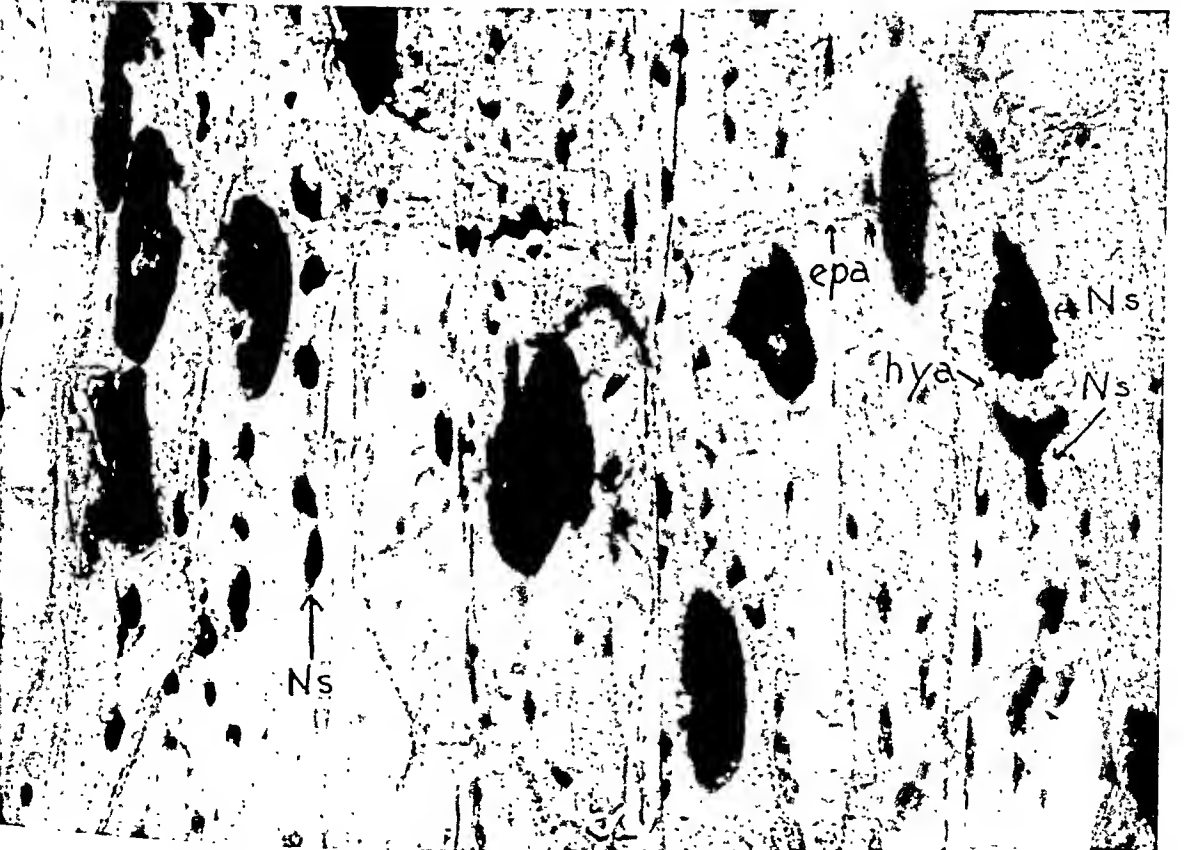
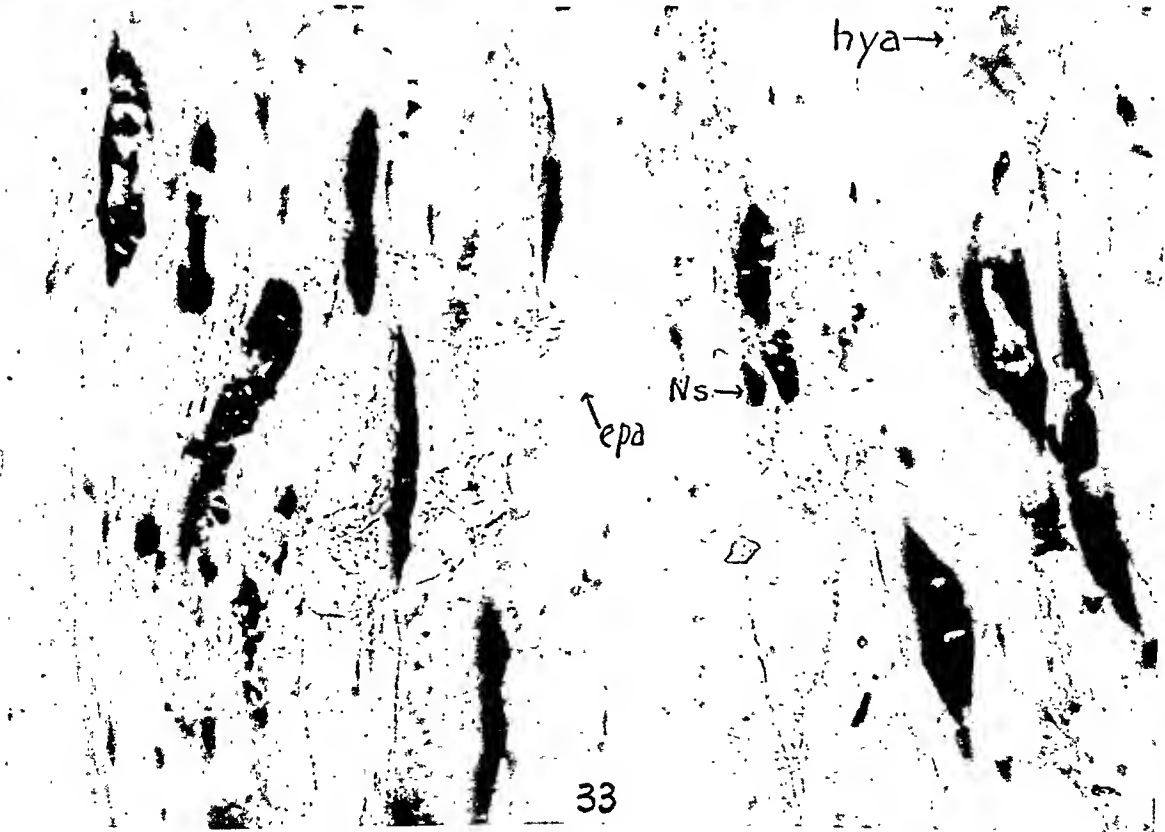
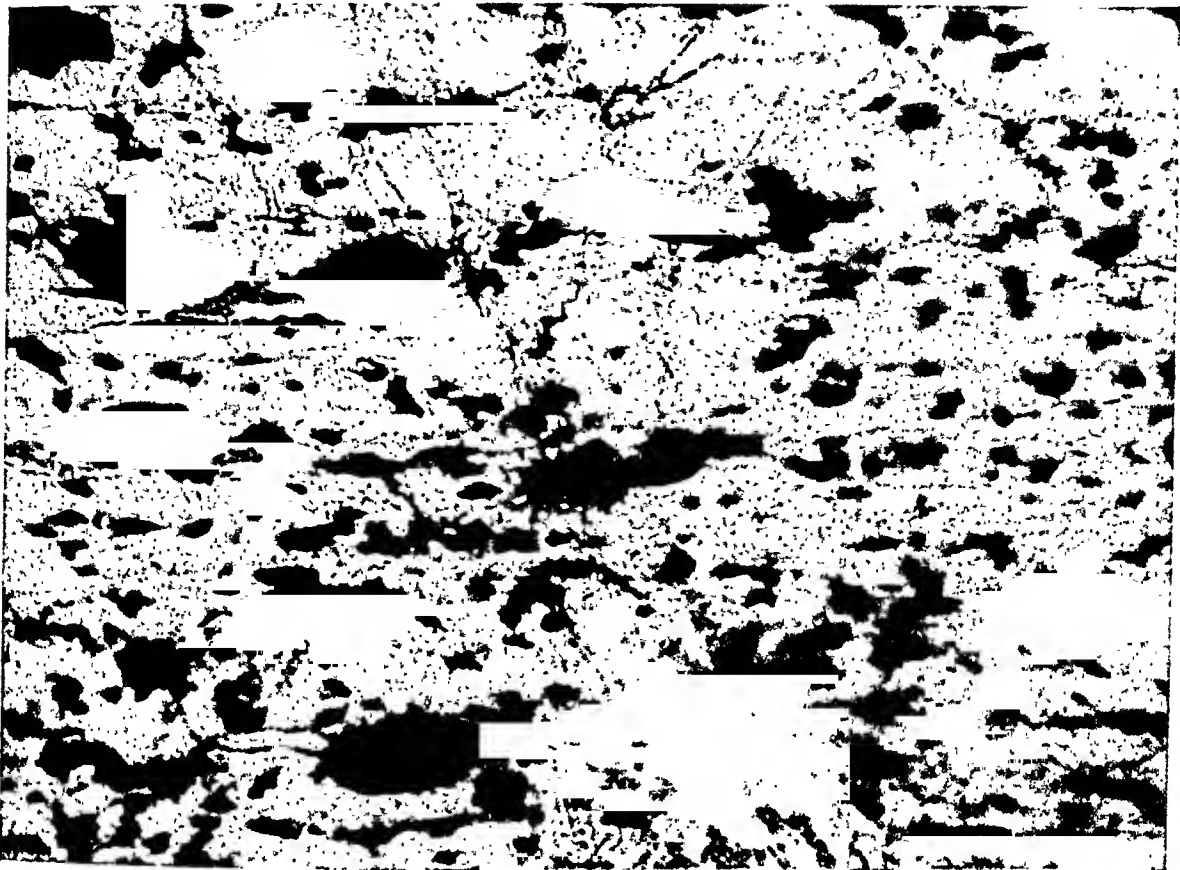


PLATE 255

FIGS. 32 and 33. Sprays of medullated nerve fibers depleted of their degenerated axonic and myelin materials, and degenerated motor end-plates surrounded by an augmented amount of substances with a strong affinity for gold in the gastrocnemius muscles of rats, 15 (Fig. 32) and 20 (Fig. 33) days, respectively, after degenerative section of the sciatic nerves. The intraperitoneal injection of d-tubocurarine chloride was followed by a short electrical stimulation of the distal segment of the cut nerve for 30 seconds at the rate of 5 per second. There is practically complete depletion from the epilemmal axons of the substances with an affinity for gold. This gold-impregnated material has been discharged in a centrifugal direction into and around the degenerated motor end-plates. This discharge has, likewise, occurred in a very profuse manner throughout the muscle fibers. There is a definite loss of the dark type of muscle fiber (for comparison with Fig. 22, normal innervation of gastrocnemius muscle). The demonstration of the pathologic structure of this abnormal transmitter substance is due to the experimental acceleration of the discharge and the lack of normal diffusion of this abnormal transmitter substance into the myoplasm of the muscle fiber. The irregular discharge of nondiffusible nervous substances into the muscle apparently prevents the appearance of the functional dark type of muscle fiber. There is a definite elongated and fusiform structure assumed by the gold-impregnated material that accumulates around the degenerated motor end-plates about the 20th day after the degenerative cut. This is a mechanical resultant of the atrophy and decrease in diameter of the denervated gastrocnemius muscle fibers. This atrophy of the muscle fibers mechanically molds the liquid or semiliquid neurogenic materials discharged, at the terminals of the degenerated motor nerve, into the muscle fibers. The demonstration of the pathologic structure of the normal or abnormal transmitter substance may be accomplished best, experimentally, by accelerating the discharge of the axonic material into the muscle and by preventing or delaying its normal diffusion into the myoplasm.  $\times 350$ .



## PLATE 256

FIGS. 34 and 35. Sprays of medullated nerve fibers and terminal motor end-plates from the relatively normal gastrocnemius muscle of a rat killed by ether. The dark and light muscle fibers are evident. The dark muscle fibers contain more material that has an affinity for gold than the light muscle fibers. The dark, anisotropic, transverse bands in the dark muscle fibers are usually broader and darker than those in the light fibers. On the other hand, the light, isotropic, transverse striations in the light muscle fibers are usually not only broader than the light spaces in the dark muscle fibers, but there is less material that has an affinity for gold in the light spaces of the light muscle fibers than in corresponding cross striations of dark fibers. There are multiple gradations in the width and capacity to take gold of the cross striations. The motor end-plates in the dark, narrow muscle fibers usually possess coarse fronds or knob-like terminals. These fronds may or may not be surrounded by light halo-like spaces, but they are usually surrounded by more dark, granular material of the sole plate of Kühne than in the sole of the expanded end-plates in light muscle fibers. The granules of the sole plate are derived from two sources: from the granular transformation and permeability of the terminals of the hypolemmal axons, and from the nuclei of the sole plate. This granular material of the sole plate normally diffuses in a periodic manner throughout the myoplasm of the dark fiber. The motor end-plate in the light muscle fiber usually exhibits an ameboid expansion and an attenuation of the hypolemmal axons. There is, likewise, a decreased amount of the diffusible granules of Kühne around the motor end-plates of the light muscle fibers. The dark fiber may well represent the stage at the onset of muscle contraction and the light muscle fiber the phase of active contraction. It is practically impossible to catch a muscle fiber in a state of completely unstable relaxation by any histologic technic used to date. The dark muscle fiber probably represents the state nearest to that of relaxation or at the beginning of the stage of contraction of the myoplasm produced by the periodic diffusion of acetylcholine. The dark muscle fibers in the teased specimens have a strong affinity for gold and are stained deep red, blue, or purple. The light muscle fibers have a decreased affinity for gold or are entirely devoid of this staining property, probably because of the temporary absence of chemicals that have an affinity for gold. There are multiple gradations between the hyperchrysophilous and the achrysophilous fibers.  $\times 850$ .

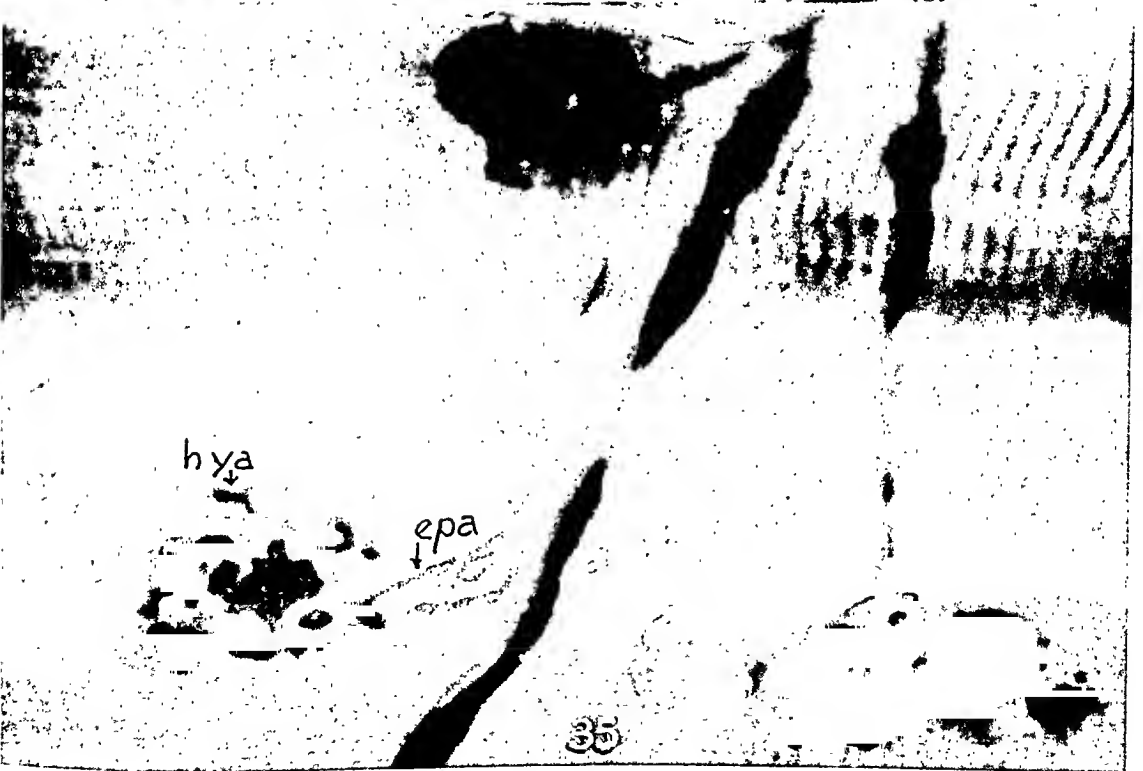
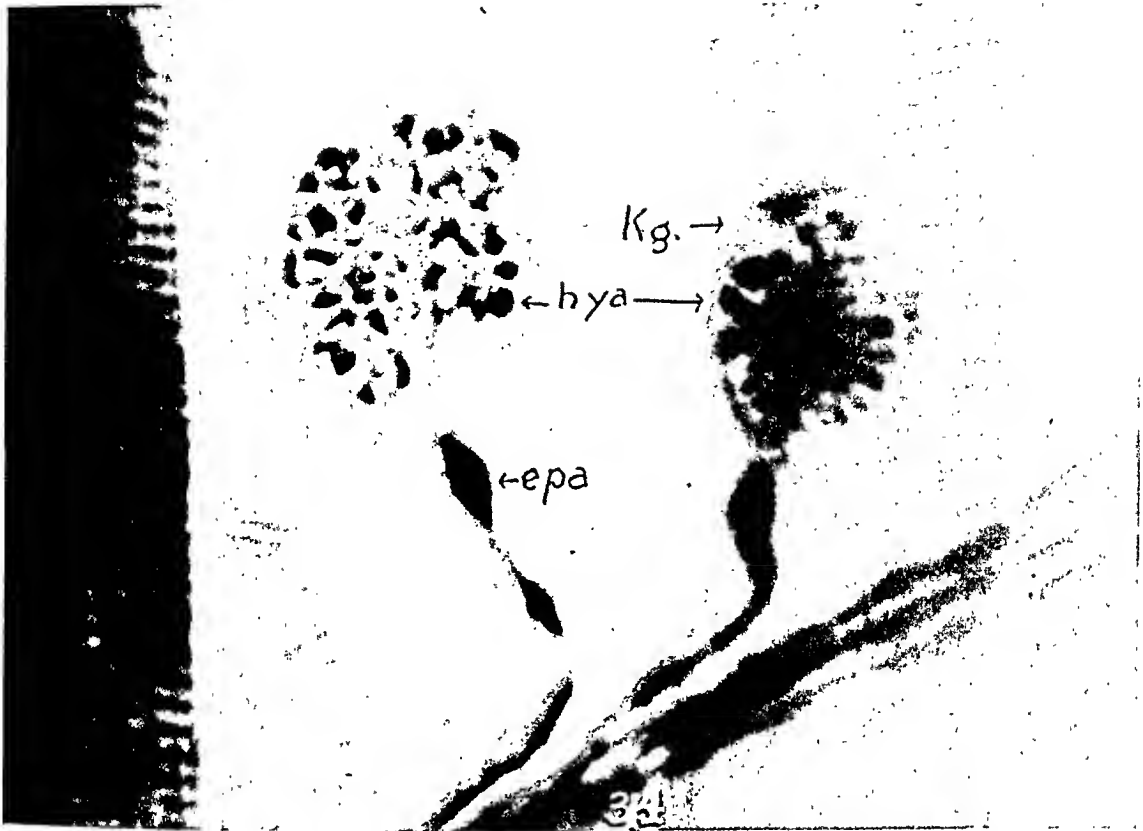
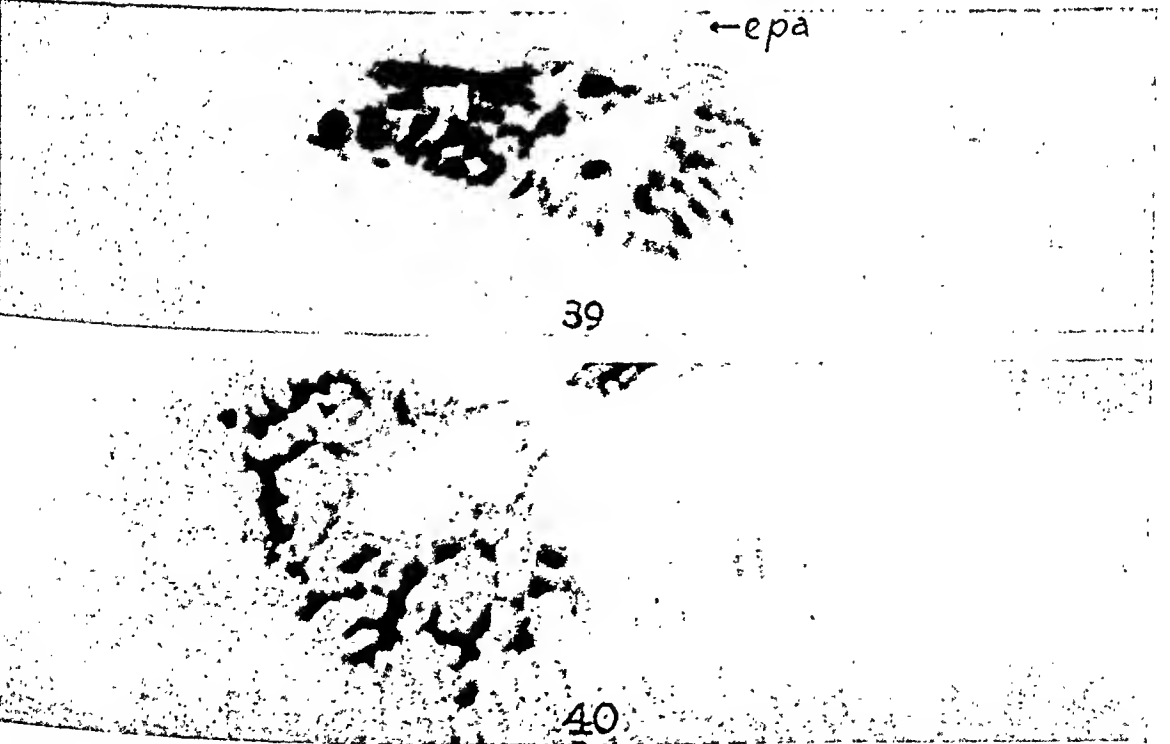
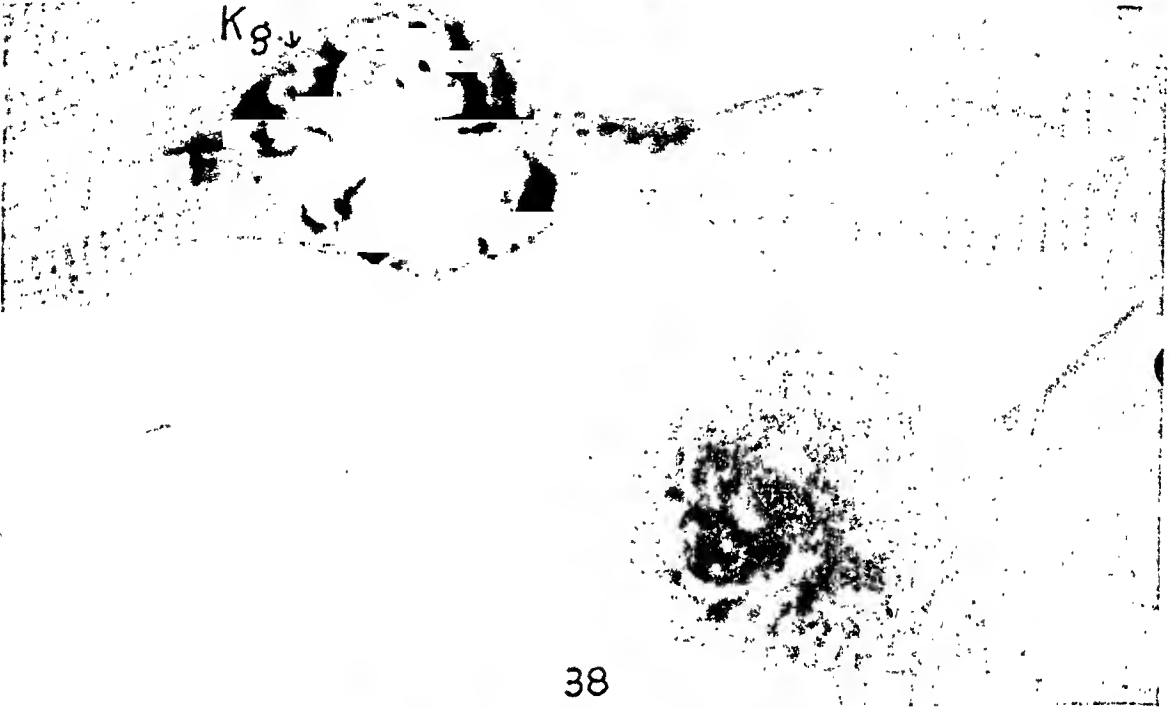
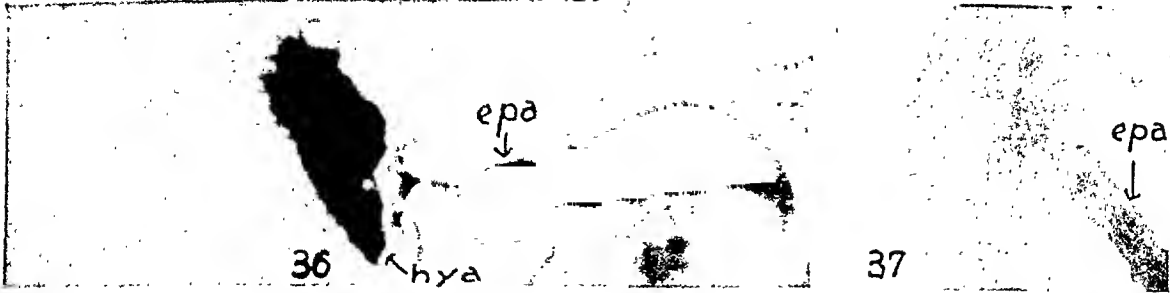


PLATE 257

FIGS. 36 to 40. Motor end-plates from the gastrocnemius muscle of a rat 24 hours after the degenerative cut of the sciatic nerve. Some retracted end-plates (Figs. 36 and 37) are associated with very few Kühne's granules but are in continuity with epilemmal axons that are enlarged and form cysts. There appears to be a temporary block of transmission of substance from the epilemmal axon to the hypolemmal ramifications in the nerve ending. Some of the end-plates are relatively normal, but in places there are relatively large, rounded, or oval thickenings, varying in number, size, and shape, which appear on the arborizations of the motor endings. These retention cysts contain materials that have a strong affinity for gold (Figs. 36, 37, and 38). Other end-plates are relatively expanded with ameboid branchings of their terminal arborizations (Figs. 39 and 40). These terminal branchings are more elongated, more narrow, and have a decreased affinity for gold as compared to the relatively retracted endings. The expanded endings, likewise, have a decreased amount of the Kühne granules of the sole plate. The direct transformation of the hypolemmal axons into granules appears to be one origin of the granular sole plate of Kühne. The retracted and expanded nerve endings, therefore, are functional types that alternate in the same muscle fiber at different times and do not represent a specific definitive type of nerve ending that is always constant in its structure.  $\times 850$ .





## PLATE 258

FIGS. 41 to 45. Motor end-plates from the gastrocnemius muscle of a rat 48 hours after the degenerative cut of the sciatic nerve. Some retracted end-plates (Fig. 41) are associated with very few Kühne's granules whereas others (Fig. 42) possess a large amount of surrounding Kühne's granules. There appears to be a cystic enlargement of both the hypolemmal (Fig. 41) and epilemmal (Fig. 42) axons in some places. These cystic enlargements contain materials that have a strong affinity for gold. In other locations of the same muscle (Figs. 43, 44, and 45) there is an expansion of the hypolemmal axons of the motor end-plate. This appears to be accomplished by an ameboid extension of the hypolemmal axons into the myoplasm. In some of these expanded motor endings (Fig. 43) there is found a peripheral disposition of Kühne's granules separated from the axons in some places by a clear, halo-like area, while in others the granules are in direct continuity with the hypolemmal axons. Certain expanded motor end-plates (Fig. 44) have an increased quantity of materials with an affinity for gold. In other locations of the same muscle (Fig. 45) the extremely attenuated and narrowed ramifications of the motor end-plate are undergoing a transformation into granules. They possess a greatly decreased quantity of materials with an affinity for gold. This morphologic change appears to be due to the direct transformation of the motor nerve endings into granules, and the discharge and periodic diffusion of these granules into the myoplasm. Under abnormal overstimulation of a continuous type due to the degenerative cut of the sciatic nerve, this results in a progressive exhaustion of the motor end-plates of their materials that have an affinity for gold. There are 15 single dark cross striations related to the retracted motor end-plate (Fig. 41) and 33 duplex striations related to the expanded end-plate (Fig. 44). It is clearly evident, therefore, that the cross striations do not form constant and fixed relations with the motor end-plate. If they did, they would be mechanically greatly separated during the period of the expansion of the motor end-plate. The cross striations periodically appear and disappear depending upon capillary chemical changes in composition and concentration at the neuromuscular apparatus. This morphologic evidence is not consistent with the theory that there is a constant number of units of sarcomeres in a voluntary muscle fiber or that the *sine qua non* of striated muscle is the continuous existence of a series of so-called sarcomeres composed of ZJQJZ cross bands. These cross striations are produced by the appropriate microcapillary chemical conditions of periodic condensation and normal diffusion of substances, involving the Liesegang phenomenon. They do not depend upon constant and rigid architectural design of a static structure as taught in histology.  $\times 850$ .

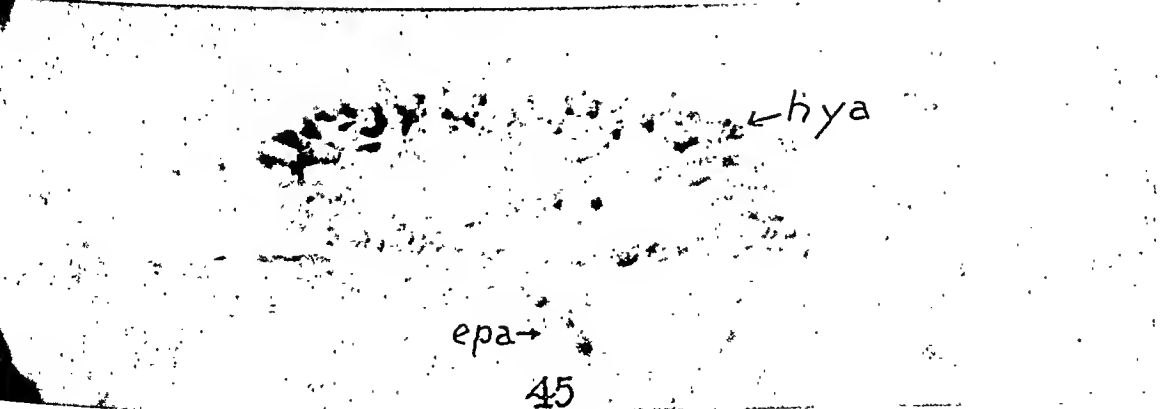
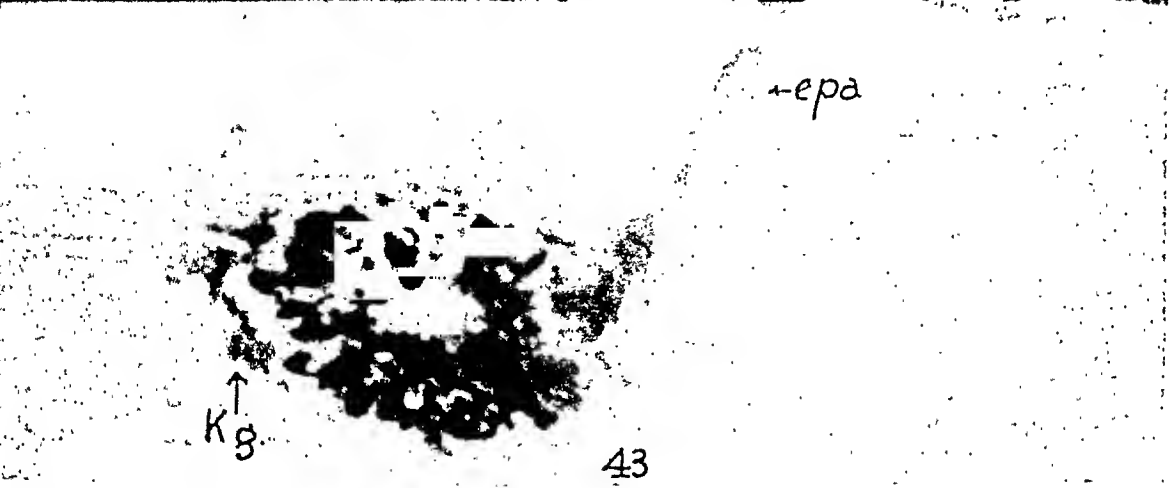


PLATE 259

FIGS. 46 to 49. Motor end-plates from the gastrocnemius muscles of rats 24 hours (Figs. 48 and 49) and 48 hours (Figs. 46 and 47), respectively, after the degenerative cut of the sciatic nerves. Some retracted end-plates (Fig. 46) contain a large central island composed of materials that have a strong affinity for gold. Some of the hypolemmal axons have undergone granular transformation and are continuous with the streaming of granules from the sole plate of Kühne out into the myoplasm. Other arborizations of the motor end-plates are enlarged and form cysts containing material with a strong affinity for gold (Fig. 46). In other locations of the same muscle practically all of the hypolemmal axons have undergone a granular transformation. These granules are directly continuous with, and are discharged into, the myoplasm (Fig. 47). Among the granules of the transformed motor end-plates will be found clear, rounded, and oval spaces which contain the nuclei of the sole plate. The epilemmal axons have an irregular contour. In other places there is an enormous enlargement of the motor end-plates by ameboid expansion (Figs. 48 and 49). These expanded terminals have relatively large, rounded, or oval thickenings, which vary in number, size, and shape. These enlargements which appear on the arborizations of the motor endings have a strong affinity for gold. There is no definite granular sole plate but there is evidence, in certain places, of the direct transformation of the end arborizations into granules which diffuse into the myoplasm. In some retracted (Fig. 46) and expanded (Figs. 48 and 49) end-plates the related cross striations of the muscle fibers are replaced by granules. It is clearly evident that more cross striations would be related to the expanded than to the retracted nerve ending if the inconstant cross striations were present.  $\times$  850.

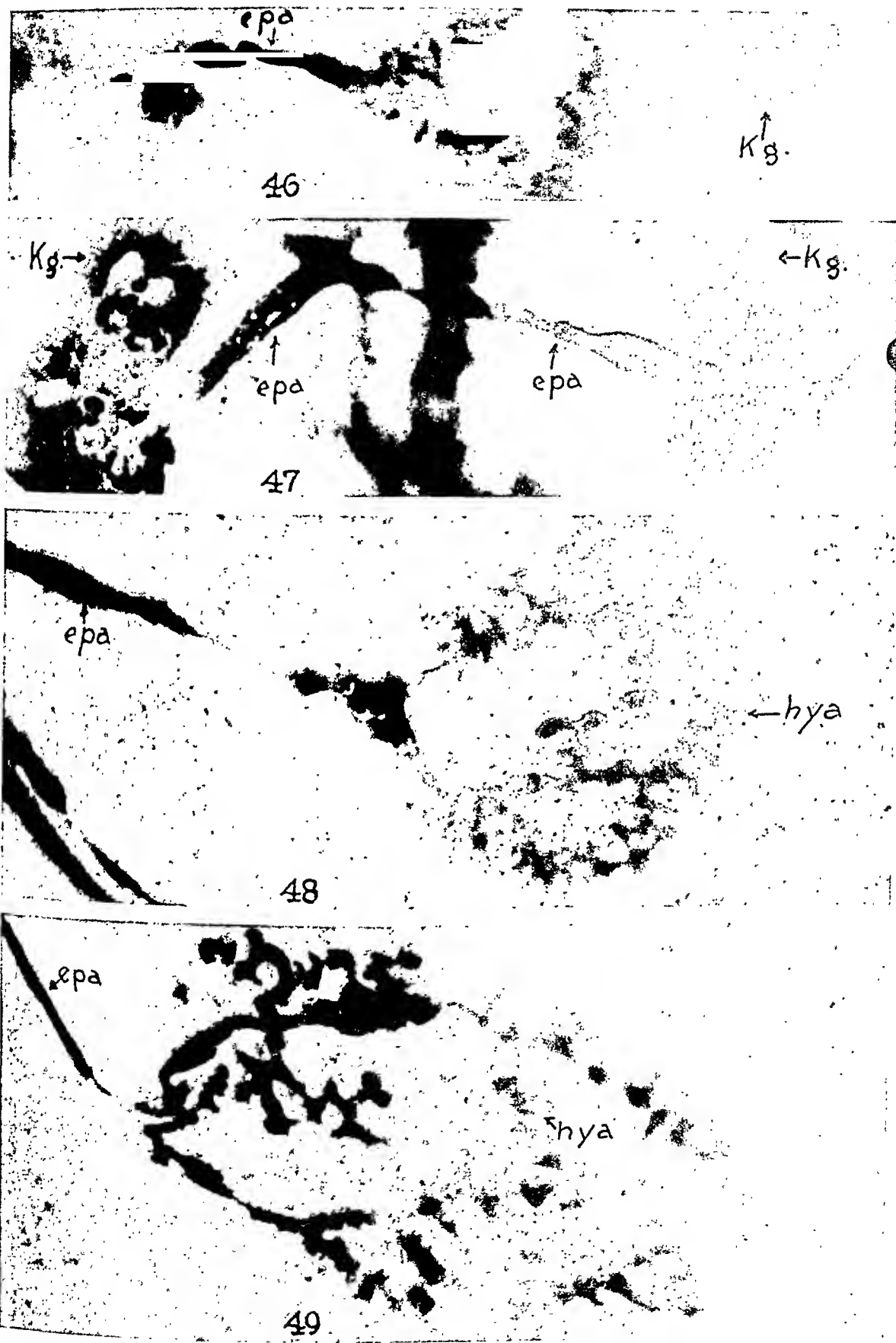
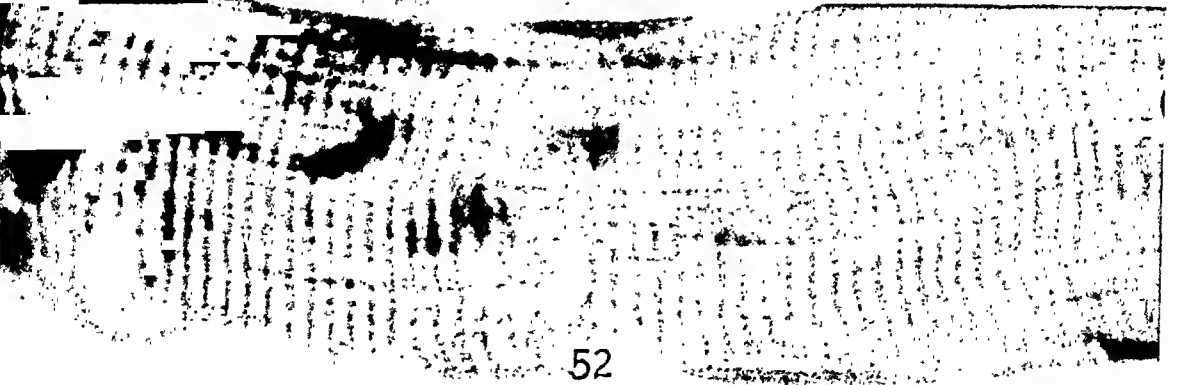
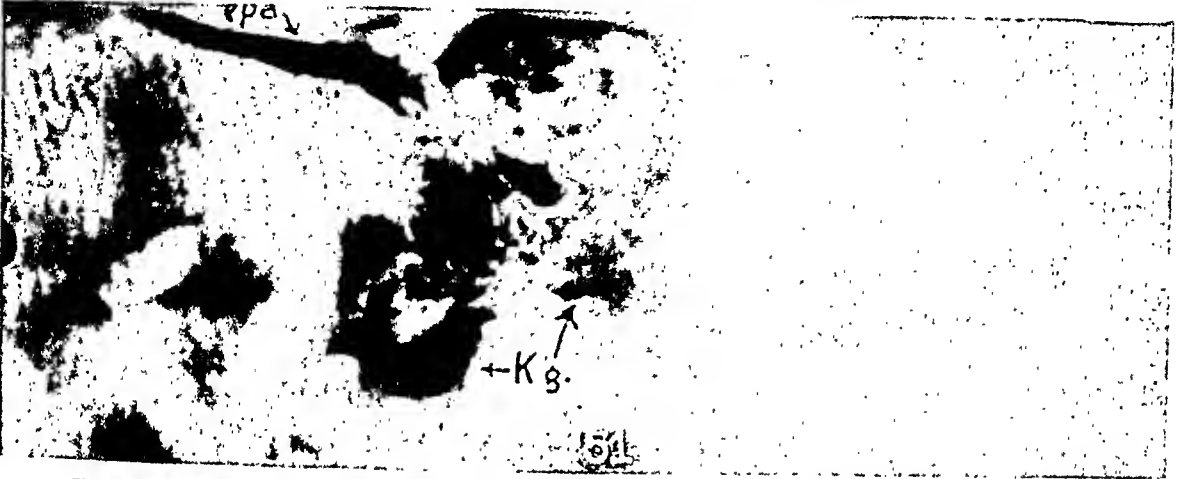
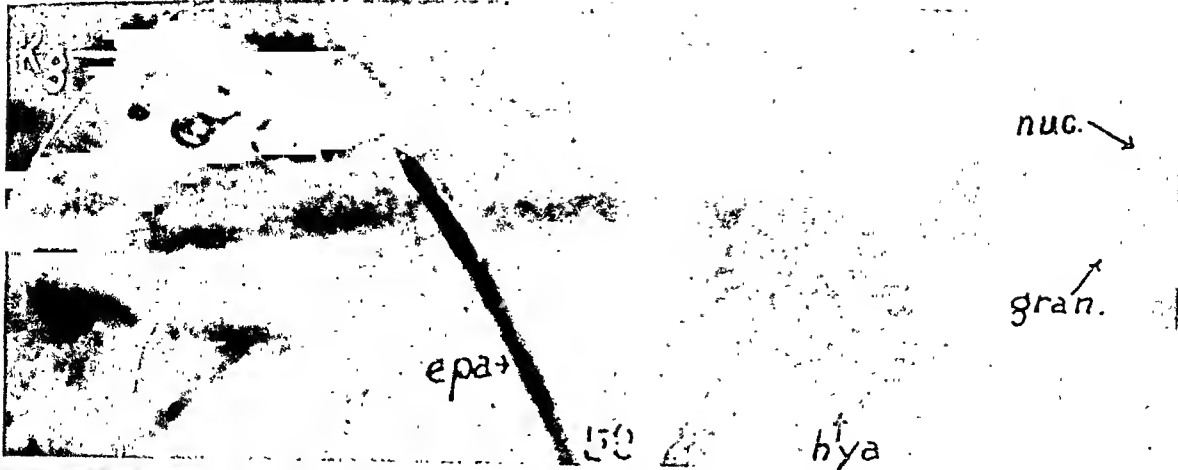


PLATE 260

FIGS. 50 to 53. Motor end-plates from the gastrocnemius muscle of the rat 72 hours after the degenerative cut of the sciatic nerve. Progressive stages are demonstrated in the direct transformation of the hypolemmal axons of the motor end-plates into granules (Figs. 50 and 51). Practically all of the end arborizations of the motor end-plate (Fig. 51) have undergone granular transformation. The dark islands with a strong affinity for gold in this end-plate represent aggregations of granules discharged from the hypolemmal axons by their direct granular transformation. The clear, rounded, and oval spaces both within the region of the end-plate and out in the myoplasm are occupied by nuclei. Each nucleus has a clear, oval, or rounded space, and is surrounded by a rim of granules that varies in thickness and density. The subsarcolemmal nuclei have become larger than those within the granular sole plate. The increase in granules around the bloated nuclei of the myoplasm may be correlated with a temporary increase of the enzyme, cholinesterase, because of failure of neutralization due to diminution of nondiffusible acetylcholine at this time. The nuclei of the sole plate appear atrophic in comparison with those in the myoplasm of the same fiber. They are either fusiform, rounded, or oval, and with the gold technic form clear spaces surrounded by granules. The granular material both in the sole plate of Kühne and out into the myoplasm is contributed from two sources: the granular transformation of the hypolemmal axons of the motor end-plate, and the nuclei of the sole plate and the sarcolemmal nuclei of the muscle (Figs. 52 and 53).  $\times 850$ .



## PLATE 261

FIGS. 54 to 57. Motor end-plates in the gastrocnemius muscles of rats 72 hours (Figs. 54 and 55), 5 days (Fig. 56), and 20 days (Fig. 57), respectively, after the degenerative cut of the sciatic nerves. Progressive stages are demonstrated of the direct transformation of the hypolemmal axons of the motor end-plates into granules (Figs. 54 and 55). At the fifth day, in many places, the granules of the hypolemmal axons and of the sole plate of Kühne have disappeared by diffusion into the myoplasm (Fig. 56). This depletion of the epilemmal axons of their motor end-plates does not remain constant during the process of wallerian degeneration of the neuromuscular apparatus. At periodic intervals the epilemmal axon discharges the degenerated material into the region of the degenerated motor end-plates. The nuclei of the motor end-plate and sole plate of Kühne become increasingly visible and aggregated due to the accumulation of unused granules around their periphery. Some of these granules are the products of nuclear activity and other granules in this region are discharged from the degenerating epilemmal axon into the region of the degenerated hypolemmal axons. With complete exhaustion of the degenerated material in the epilemmal axons the nuclei still remain visible by the gold technic as clear, rounded, or oval spaces surrounded by granules discharged by the nuclear activity.  $\times 850$ .

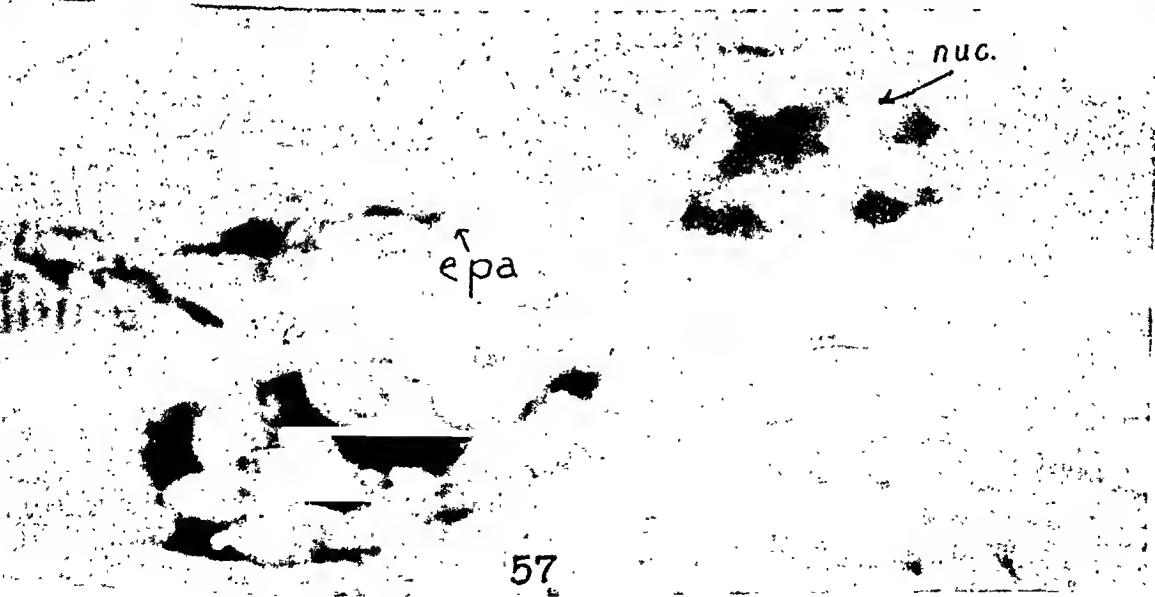
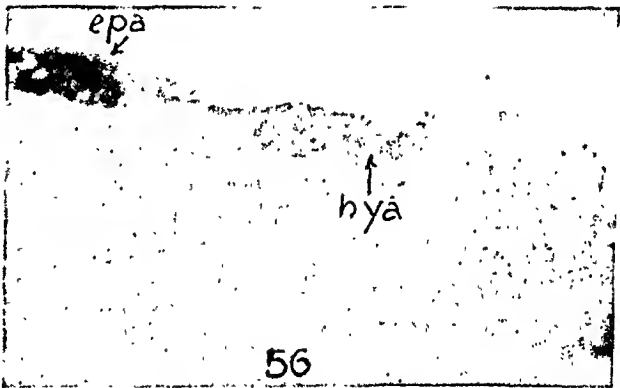
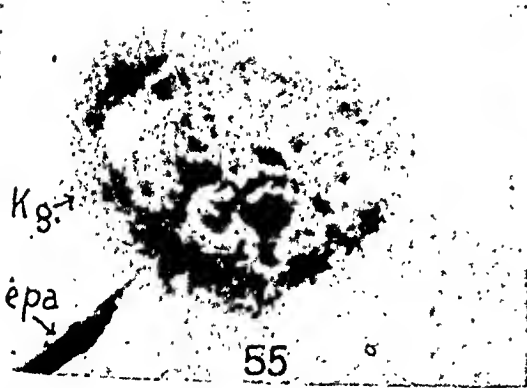
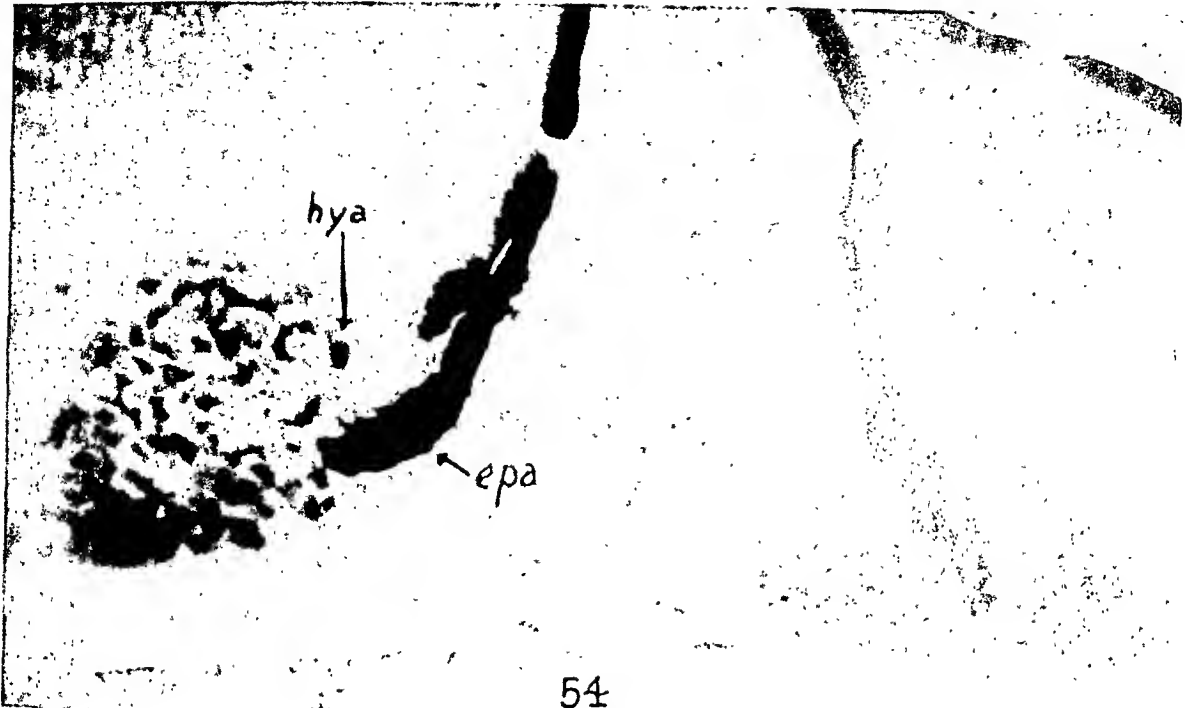




PLATE 262

FIGS. 58 to 62. Motor end-plates in the gastrocnemius muscles of rats 5 days (Figs. 58 and 59) and 10 days (Figs. 60, 61, and 62), respectively, after the degenerative cut of the sciatic nerves. The intraperitoneal injection of d-tubocurarine chloride was followed by a short electrical stimulation of the distal segment of the cut sciatic nerve for 30 seconds at the rate of 5 per second. The degenerated axonic and myelin materials discharged into the zone of the degenerated motor end-plates have a pleomorphic arrangement: unipolar (Figs. 58 and 59), bipolar (Fig. 61), and multipolar or completely circumferential (Figs. 60 and 62). This gold-impregnated material has been discharged into and around the degenerated motor end-plates in a centrifugal direction from the epilemmal axons. There is a progressive depletion of the material with an affinity for gold from the epilemmal axons during this period of discharge into the region of the degenerated motor end-plates. There is unimpeachable evidence that the gold-impregnated material is initially in direct anatomic continuity with the structures that innervate the muscle. In one end-plate (Fig. 59) the discharged neurosome is in direct anatomic continuity with the hypolemmal axon of the motor end-plate. It forms what has been designated by some neuroanatomists as an ultraterminal nonmedullated branch of the motor end-plate that ends in the same muscle fiber in an enlarged spherical or oblong terminal. This ultraterminal branch of the motor end-plate, however, is merely the initial phase of the discharge of materials from the nerve terminal when anatomic continuity is still maintained. It is the product of the abnormal discharge of substances by abnormal stimulation from the motor ending and not a specific type of morphologic ending as claimed by some observers.  $\times 850$ .

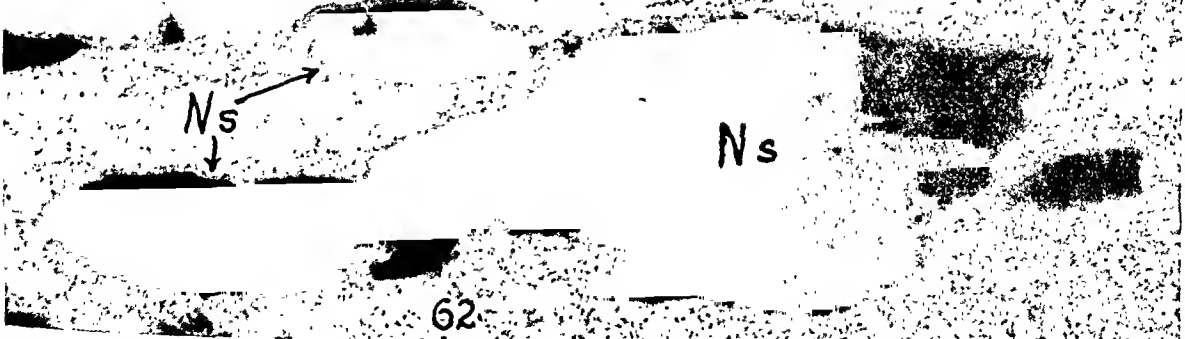
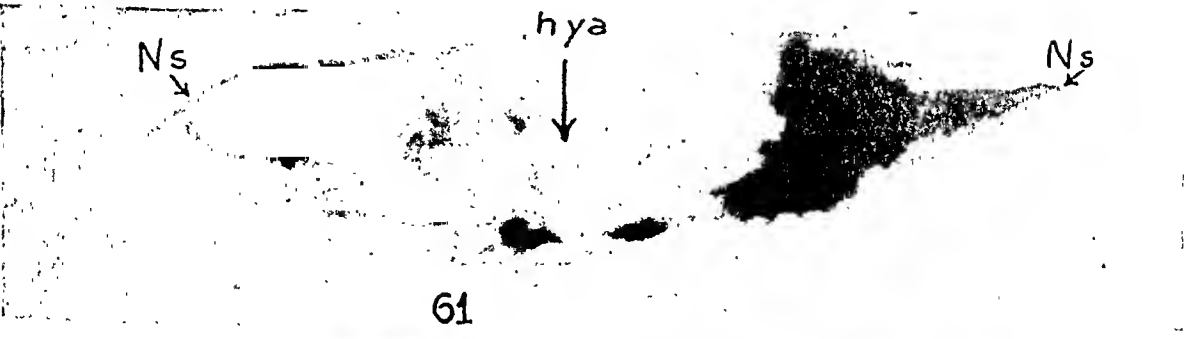
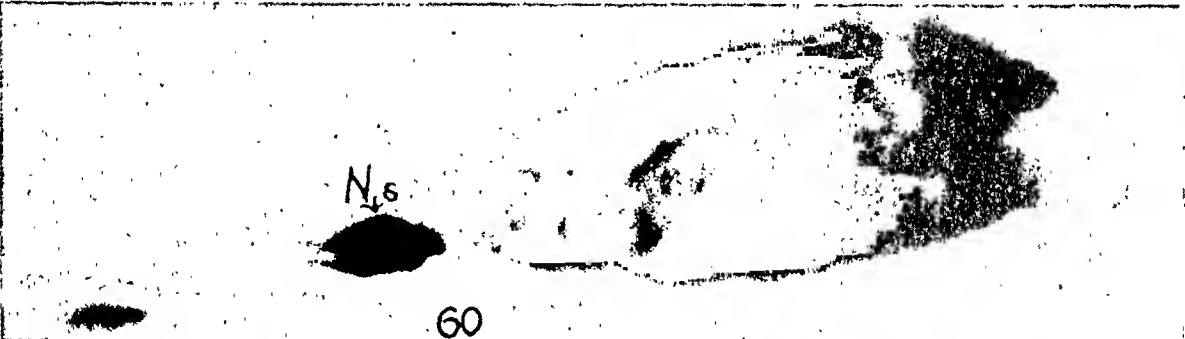
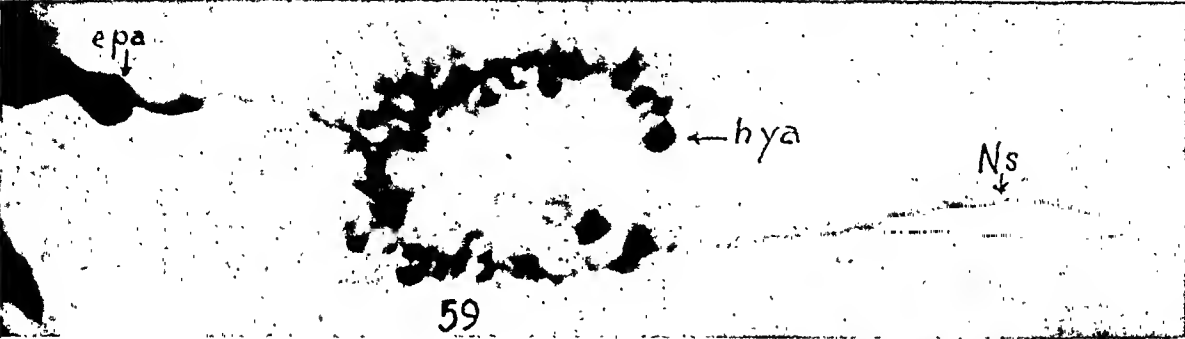
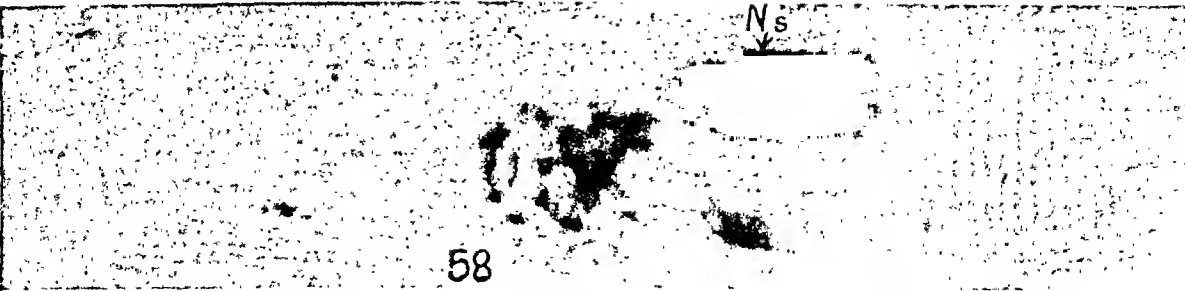


PLATE 263

FIGS. 63, 64, and 65. Motor end-plates in the gastrocnemius muscle of the rat 5 days after the degenerative cut of the sciatic nerve. The intraperitoneal injection of d-tubocurarine chloride was followed by a short electrical stimulation of the distal segment of the cut nerve for 30 seconds at the rate of 5 per second. This gold-impregnated material has been discharged into and around the degenerated motor end-plates in a centrifugal direction from the epilemmal axons. The central region of the degenerated motor end-plates may be clear, surrounded by a thick rim of material that has a strong affinity for gold (Fig. 65), or there may be a dark center with radiating dark streaks like a wheel with hub and spokes, constituting the arrangement of the material discharged from the terminal of the depleted epilemmal axon (Fig. 63). To the right of one end-plate (Fig. 64) there is direct anatomic continuity between the gold-impregnated material surrounding the degenerating end-plate and the discharge of a fusiform neurosome out into the myoplasm of the muscle fiber.  $\times 850$ .

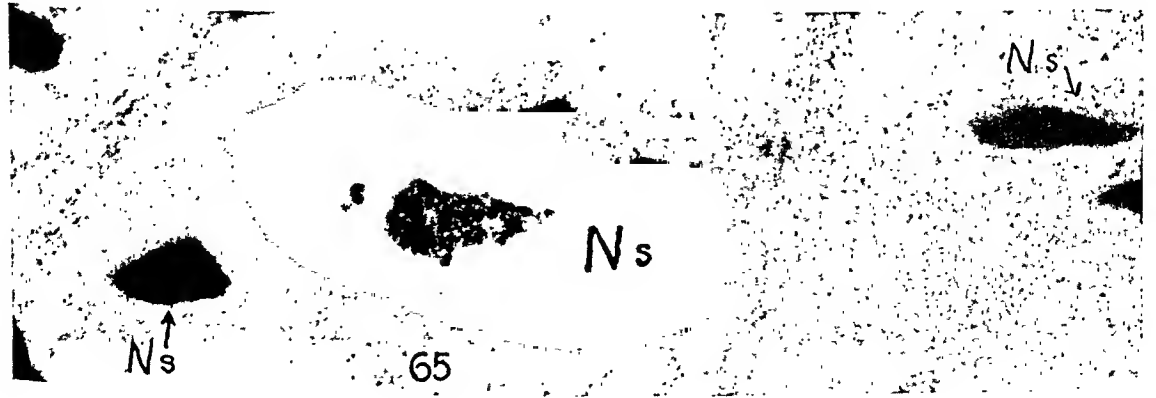
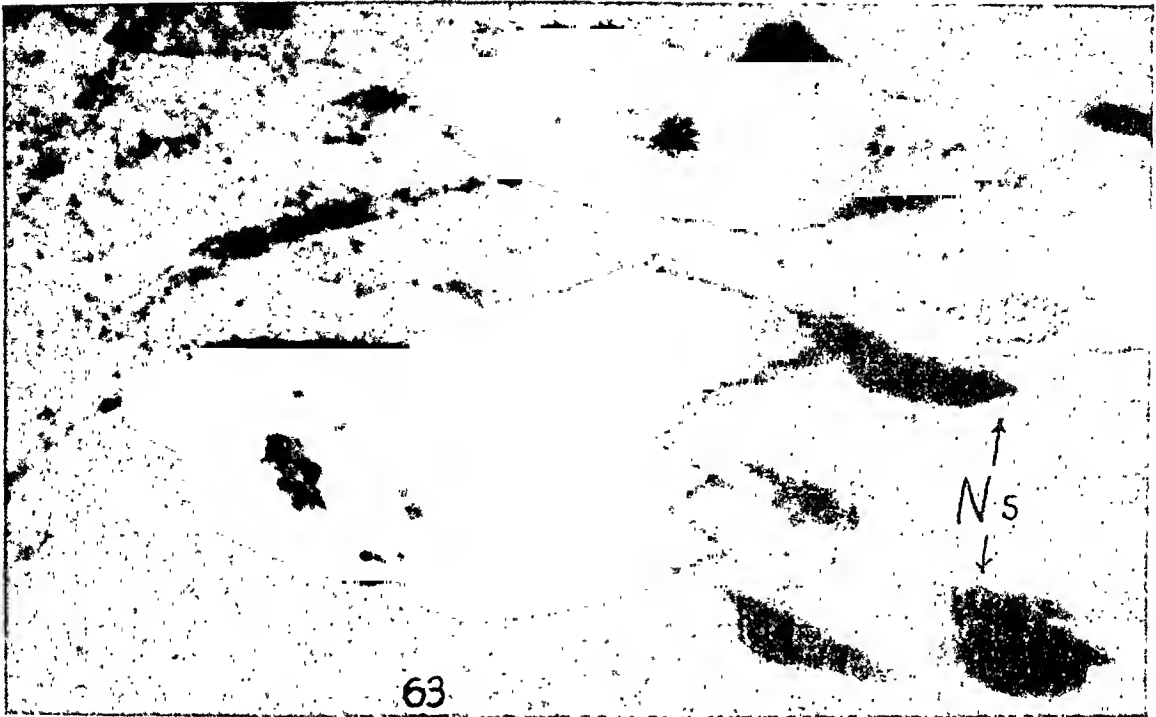


PLATE 264

FIGS. 66 to 70. Degenerated motor end-plates in the gastrocnemius muscle of the rat 15 days (Figs. 66 to 68) after the degenerative cut of the sciatic nerve. The intraperitoneal injection of d-tubocurarine chloride was followed by a short electrical stimulation of the distal segment of the cut nerve for 30 seconds at the rate of 5 per second. Curare and electrical stimulation were not used in the animal from which the motor end-plates were obtained (Figs. 69 and 70) 15 days after the degenerative cut of the sciatic nerve. It is clearly evident that under the conditions of the experiment there was an accelerated discharge of increased quantities of substances with an affinity for gold from the epilemmal axons into and about the regions of the degenerated motor end-plates (Figs. 66 to 68) in comparison with the condition in the end-plates after simple section of the sciatic nerve (Figs. 69 and 70). Thus, by experimental means, there may be an acceleration of the discharge of abnormal neurogenic substances from the motor nerve endings into the muscle fiber.  $\times 850$ .



PLATE 265

FIGS. 71 to 75. Gastrocnemius muscle fibers from an etherized rat 15 days after the degenerative cut of the sciatic nerve. The intraperitoneal injection of d-tubocurarine chloride was followed by electrical stimulation of the distal segment of the cut nerve for 30 seconds at the rate of 5 per second. The discharged neurosomes (Ns) found in the myoplasm are pleomorphic and hyperchromatic for gold in comparison with the cross striations of the related muscle fibers in which they are found. These neurosomes may be fusiform (Figs. 71 and 72), irregularly oblong with one end tapering (Figs. 73 and 74), arrow-headed in shape, or like rounded droplets that vary in size (Fig. 75). The droplets form series of either single drops widely separated from one another or a closely related series composed of 2 to 10 droplets. The droplets in the middle of the series are usually larger than the terminal ones. Such series of droplets form an irregular fusiform structure separated by clear spaces. The large fusiform neurosomes may have edges that are either serrated (Fig. 71) or festooned (Fig. 72). The sharp projections arranged like saw teeth around the edges of the neurosome may or may not be in direct alignment with the dark cross striations of the muscle fiber. These neurosomes undergo a granular dissolution, and the granules become incorporated into the myoplasm, and are then aligned with the cross striations of the muscle fiber. These neurosomes are periodically discharged from the motor endplates into the muscle fiber. During degeneration they are more persistent than those produced by the artificial overstimulation of normal muscle or those produced by neurogenic shock.  $\times 850$ .

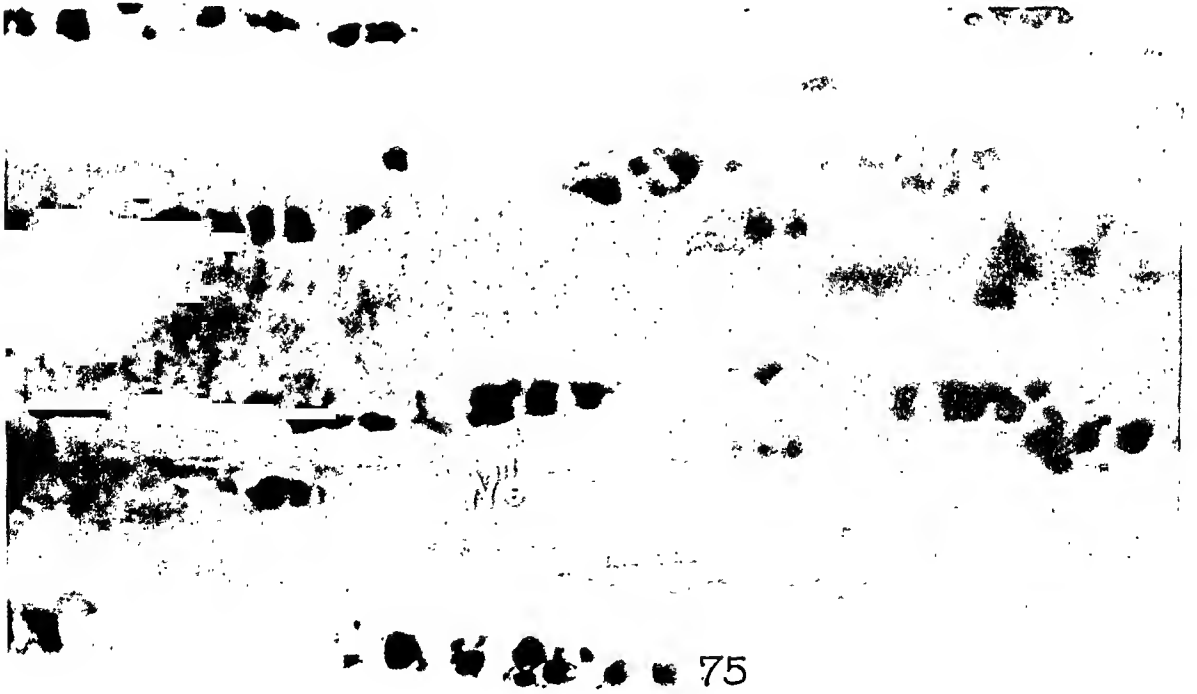
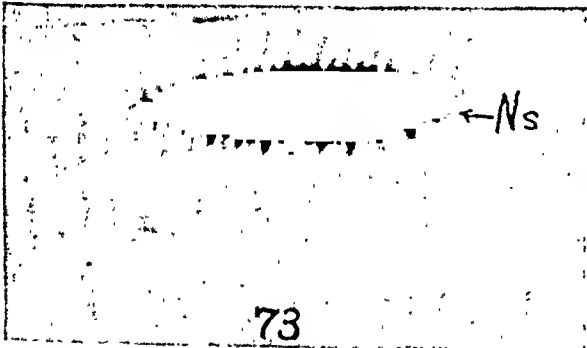
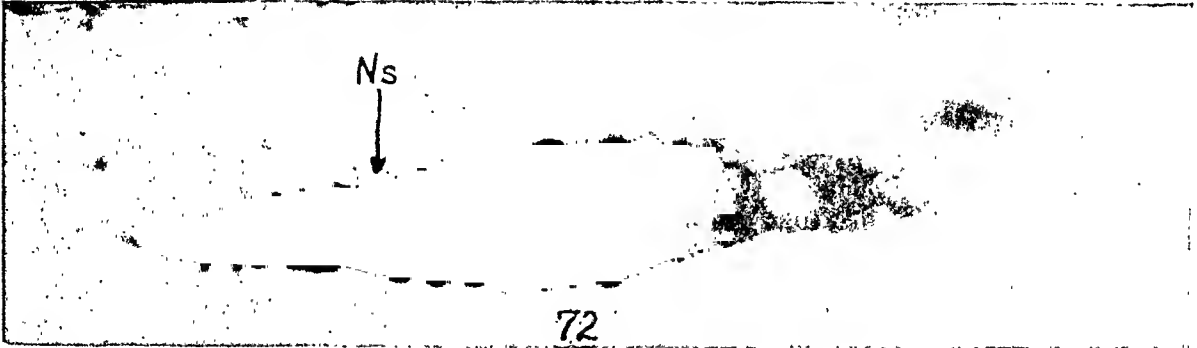
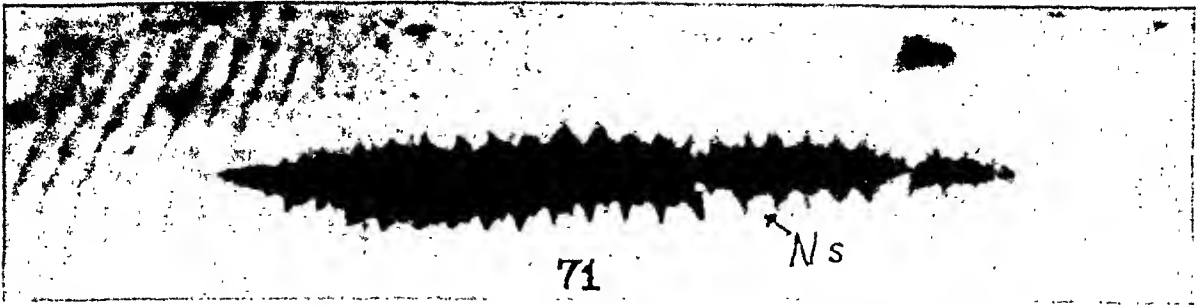




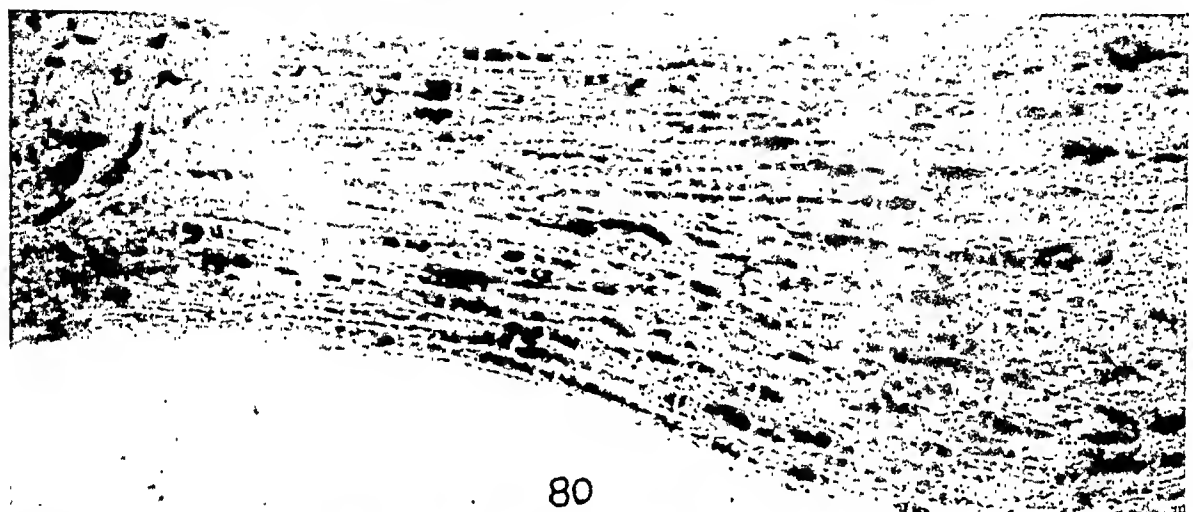
PLATE 266

FIGS. 76, 77, and 78. Gastrocnemius muscle fibers from an etherized rat 14 days after the degenerative cut of the sciatic nerve. The intraperitoneal injection of d-tubocurarine chloride was followed by electrical stimulation of the distal segment of the cut nerve for 30 seconds at the rate of 5 per second. In a few locations giant muscle fibers (Gmf., Fig. 76) are found. Their diameter is increased three to four times above that of the closely related muscle fibers. These giant muscle fibers are densely impregnated with gold, indicating an abnormal discharge and accumulation of increased quantities of abnormal axonic material in the fiber under the conditions of the experiment. The cross striations of the giant muscle fiber are seen only at its edges because of the dense opacity produced by the discharged nervous material. There is a definite streamlined effect produced by certain large neurosomes (Ns) upon the cross striations of the muscle fiber. This streaming effect of the neurosomes discharged into the myoplasm is detected by the altered arrangement of the cross striations in the muscle fiber (Fig. 77). Certain large muscle fibers (Fig. 78) are not so densely packed with neurosomes as those of the giant muscle fibers (Fig. 76). The neurosomes are clearly evident as gold-impregnated bodies intermingled with the cross striations. In some locations these neurosomes are dense and opaque, while in other places, where they are undergoing granular liquefaction, they are light and cross-striated in appearance. Some of the muscle fibers in close proximity to those containing neurosomes do not possess these bodies in their myoplasm (Fig. 78). The muscle fibers without discharged neurosomes are more lightly impregnated with gold than those that possess them. Figure 76,  $\times 200$ ; Figure 77,  $\times 400$ ; Figure 78,  $\times 850$ .



PLATE 267

FIGS. 79 to 82. Branches of the distal stump of the sciatic nerve innervating the gastrocnemius muscle 14 days after the degenerative cut of the nerve. The progressive degeneration of the nerve from its proximal to its distal end is evident. The branch of the nerve just before entrance into the gastrocnemius muscle is completely depleted of the degenerated axonic and myelin substances that have an affinity for gold (Fig. 79). At a slightly more distal point in the same nerve, the gold-impregnated material is found in variable amounts in different axis-cylinders which are fragmented into rounded, oval, or fusiform bodies with a strong affinity for gold (Figs. 80 and 81). Just before the nerve gives rise to branches that directly innervate the muscle, there is again a depletion from the axis-cylinders of material that has a strong affinity for gold (Fig. 82). Only in widely scattered areas are five bodies found that have an affinity for gold. The epilemmal axons of a cut nerve that innervate the muscle are periodically engorged and depleted of the degenerated and fragmented axonic and myelin materials. This rhythmic discharge of degenerated nervous material into the muscle continues in a progressive and periodic manner and in a centrifugal direction until all of the degenerated material is discharged centrifugally into the muscle.  $\times 250$ .





XIV. EXPERIMENTAL ATHEROMATOSIS IN MACACUS  
RHESUS MONKEYS \*

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Little information is available on the occurrence of "spontaneous" arteriosclerosis in primates. Fox<sup>1</sup> found arteriosclerotic lesions of atheromatous or sclerotic type in only 8 of 796 monkeys studied, and concluded that arterial disease is not a prominent process in the species used. None of the affected animals was a rhesus macaque so commonly used in laboratory work. Kawamura's efforts<sup>2</sup> to produce cholesterol atheromatosis experimentally in 3 monkeys of the species *Macacus fuscatus* gave a negative result. One of these monkeys received 2 egg yolks daily for 150 days, while the other 2 were fed a total of 1,350 gm. and 1,650 gm. of anhydrous wool fat, respectively, within 300 days. Corwin<sup>3</sup> noted in 1938 that all attempts to elicit the condition known as cholesterol arteriosclerosis in monkeys were unsuccessful.

In a critical evaluation of these observations consideration must be given to the fact that monkeys in general are herbivorous animals, which, under normal dietary conditions, show a low tendency toward arterial cholesterol deposition. Only rabbits, when exposed over prolonged periods to an abnormal and excessive intake of cholesterol causing a considerable rise in the level of their blood cholesterol, have so far developed a cholesterol atheromatosis (Hueper<sup>4</sup>). Ssolowjew<sup>5</sup> reported, moreover, the occurrence of lipid deposits in the aortic intima and media of suckling rabbits, which he attributed to the exclusive milk diet consumed by the rabbits during their early life and which he compared with the lipid spots often seen in human babies for the same reason.

In view of the fact that primates are phylogenetically closer to man than any other species and that *Macacus rhesus* monkeys are extensively used in laboratory work, it seemed to be pertinent to attempt once more to produce atheromatosis in these animals, utilizing the observations made in suckling rabbits in the experimental conditions to be employed.

## EXPERIMENTAL PROCEDURE

Two young *Macacus rhesus* monkeys, born in the Warner Institute for Therapeutic Research, were used in the experiments. One was 5 months old and the second was 6 months old at the start of the experiment. Both had just been weaned and had been placed on a diet

\* Received for publication, November 26, 1945.

consisting of carrots, apples, oranges, and bread, with a daily supplement of approximately 90 cc. of milk into which one fresh egg was beaten and which was divided between the two monkeys. The male monkey, which weighed 1.4 kg. at the start of the experiment, in addition received daily by mouth 10 cc. of a 2.5 per cent solution of cholesterol in Mazola oil. The solution was injected into an orange or soaked into bread so as to make it more palatable. After 18 weeks on this management the daily dose of 2.5 per cent cholesterol in oil was raised to 15 cc. This dose was maintained for 20 months, when the animal at the age of 2 years and 5 months was sacrificed by the intracardial injection of 20 cc. of a 4 per cent formaldehyde solution.

The female monkey, which weighed 900 gm. at the start of the experiment, was given 5 cc. of cholesterol-oil daily for a period of 5 months. The dose was then raised to 10 cc. and maintained at this level for the remaining 8 months, at the end of which time the animal, when 18 months old, was sacrificed in the manner described. Both monkeys showed a steady gain in weight during the observation period. The male monkey weighed, at the end, 3.1 kg., whereas the female monkey weighed 1.4 kg. Their appetites were good throughout and they were always lively and playful while under the special dietary regime.

The autopsies showed essentially normal organs in both animals. The histological examination of the internal organs (lung, heart, liver, spleen, pancreas, adrenal, kidney, testis, thyroid) did not reveal any abnormalities. The inferior vena cava of the female monkey had a small area in which the endothelial cells were swollen and had proliferated. The aorta of this animal exhibited similar endothelial changes in the thoracic portion (Fig. 1). In the male monkey similar reactions were more extensive and more widely distributed in the aorta. Several transverse sections showed a crowding of the endothelial cells which were swollen in places and cuboidal. In one level of the ascending part, the endothelial cells were forming a stratified coat of slender cylindrical cells (Fig. 2). The aortic branches and the large and small arteries of the parenchymatous organs were normal.

The histological examination of the internal organs, the aorta and its branches of a monkey 7 months old, continuously nursed by its mother for the entire length of its life and succumbing to pneumonia, did not reveal any abnormal vascular reactions of the type described.

#### COMMENT

The observations reported indicate that *Macacus rhesus* monkeys do not readily react to a prolonged nutritional intake of excessive amounts of cholesterol with the formation of atheromatous aortic

lesions, even when exposed to such a dietary regimen during an early period of life. The endothelial aortic lesions, however, may represent proliferative responses to the cholesterol ingested, since similar endothelial reactions have been found in dogs following the repeated intravenous injection of other atheromatogenic substances, such as hydroxyethylcellulose solution (Hueper<sup>6</sup>) and after a prolonged oral administration of excessive amounts of cholesterol (Hueper<sup>7</sup>). They may thus represent early atheromatous reactions.

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[ Illustrations follow ]



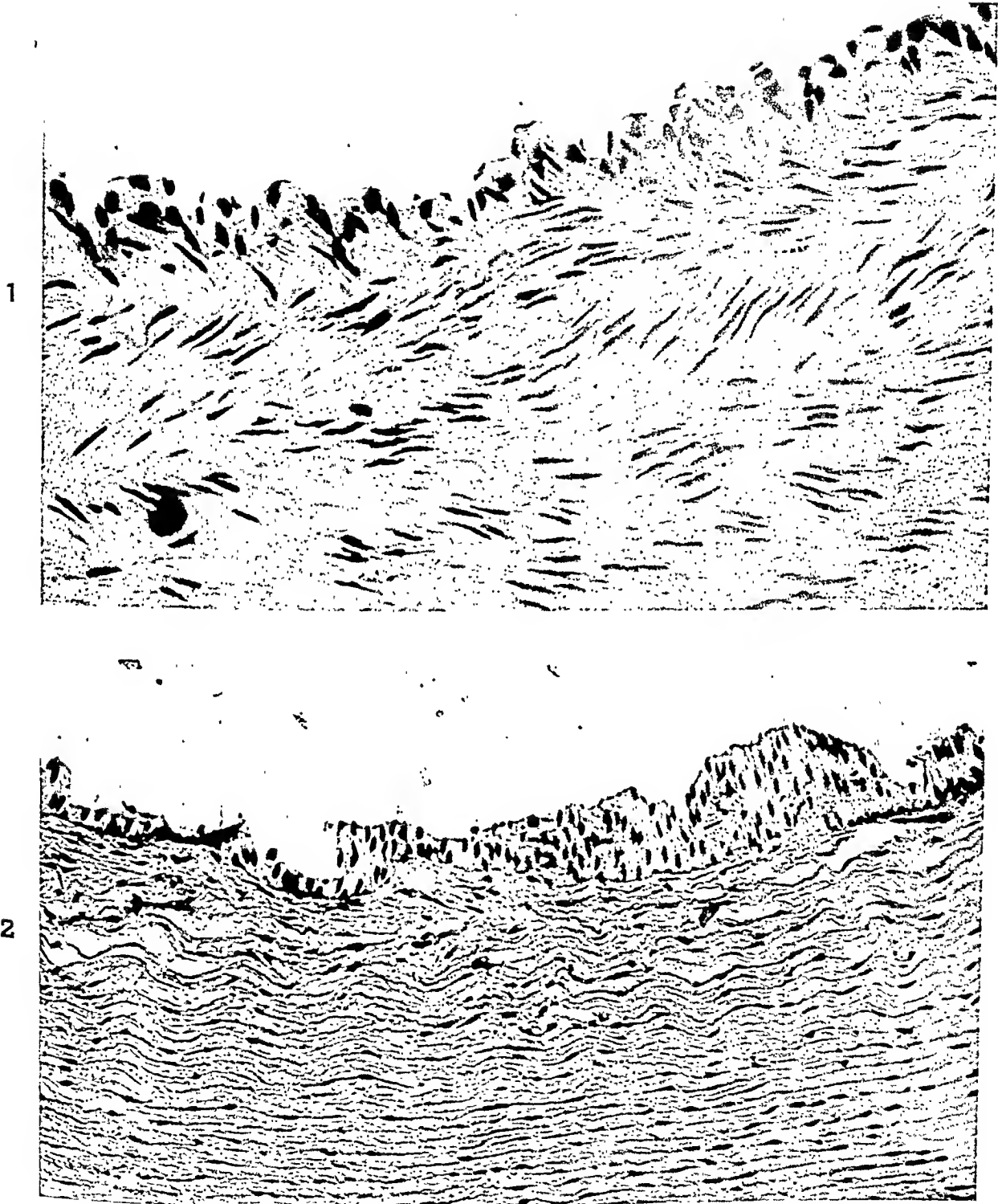
## DESCRIPTION OF PLATE

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### PLATE 268

FIG. 1. Aorta with proliferated and swollen endothelial cells.

FIG. 2. Aorta covered by a cushion of stratified, slender endothelial cells.





# METASTATIC CALCIFICATION ASSOCIATED WITH HYPERVITAMINOSIS D AND HALIPHAGIA \*

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The occurrence of metastatic calcification with various types of osseous lesions<sup>1-5</sup> and with chronic renal disease<sup>6</sup> in human pathology is well known. Experimentally, the feeding of calcium salts,<sup>7,8</sup> the administration of large doses of vitamin D,<sup>9-11</sup> and the injection of great amounts of parathyroid hormone<sup>12,13</sup> have also resulted in metastatic calcification. From the point of view of human pathology the rôle of vitamin D in the production of this lesion rests on rather scanty evidence.<sup>14-17</sup>

## HUMAN CASES OF HYPERVITAMINOSIS D FROM THE LITERATURE

Putschar<sup>14</sup> observed a male infant, 5½ months old, who received vigantol (irradiated ergosterol) for about 3 months before death. Autopsy disclosed emaciation, small adrenal glands, moderate fatty metamorphosis of the liver, a peculiar lipogranulomatous reaction in the hypodermis, and calcium deposits in the kidneys. These deposits involved mainly the epithelial cells, lumina, and basement membranes of the distal convoluted tubules, and also the stroma adjacent to them. The collecting tubules and the surrounding stroma in the pyramids were similarly involved and in much greater degree. No reactive or regressive changes were present near the calcium deposits.

Thatcher<sup>15</sup> reported the case of a male infant, 18 months old, who received irradiated ergosterol for 9 months before death. At autopsy the kidneys were enlarged, firm, and pale yellow. The medulla at the bases of the pyramids showed tiny, gritty, gray particles which microscopically were masses of calcium lying mainly in the lumina of the collecting tubules at the corticomedullary junction, with calcification of some adjacent epithelial lining cells. Calcified cells were identified in the masses of calcium, some of which were encircled by cellular fibrous tissue. The segments of tubules proximal to the calcium masses were dilated. Calcium was not found in other organs. The liver showed extensive fatty metamorphosis.

Thatcher<sup>16</sup> reported a second case in a male infant, 11½ months old, who had received much solar irradiation, two ultraviolet ray treatments, and a large amount of cod-liver oil for 4 months. Necropsy findings were practically identical with those in his first case.

Gissel and Bufo<sup>17</sup> administered large doses of dihydrotachysterol

\* Received for publication, November 12, 1945.

to two infants with meningomyelocele and hydrocephalus. One died at the age of 128 days. In addition to ascending meningitis and pyocephalus found at autopsy, the kidneys showed heavy calcium deposits in the epithelium of the cortical and medullary tubules as well as calcium casts in their lumina and scattered, finely granular calcium incrustations in the neighboring interstitial tissue. The second infant died of ascending meningitis and internal pyocephalus at the age of 54 days. The kidneys showed detached calcium casts in the cortical portions of the collecting tubules with absent or newly regenerated epithelial cells adjacent. The lungs, heart, liver, spleen, and stomach in both cases showed no calcium deposits.

Wells and Holley<sup>5</sup> thought that the reasons for metastatic calcification in the lungs, left atrial endocardium, kidneys, gastric mucosa, and hypodermis in their case of osteitis deformans were the easier mobilization of calcium from the bones in this disease, and the massive doses of viosterol given to the patient.

The observation of a recent case of metastatic calcification, in which the patient ingested vitamin D and several inorganic salts, furnishes provoking problems for solution.

### REPORT OF CASE

A cab driver, 44 years old, born in Scotland, was admitted to St. Luke's Hospital in Denver under the care of Dr. C. R. Cooper, who kindly gave permission for use of the clinical details. Since the patient was comatose, the history was obtained from his wife, who stated that for about 6 months before admission and for little understood reasons he had been taking daily doses of a vitamin D preparation as well as liberal amounts of a mildly laxative compound containing sodium sulfate, sodium bicarbonate, sodium phosphate, and sodium chloride. His wife warned him repeatedly that this was a hazardous procedure without the advice of a physician, but he persisted in his autotherapy. Two weeks before admission he had suffered from malaise, weakness, restlessness, and drowsiness. One week before he had seen an osteopath who diagnosed Bright's disease and prescribed some unknown medication. Since he did not improve and experienced periods of stupor alternating with bouts of delirium, he was admitted to the hospital on July 2, 1944, at 11:35 A.M. His temperature was 99.8° F. and his pulse was 90 beats per minute. His respirations were labored and numbered 26 per minute. Physical examination revealed stupor, pallor, a loud, blowing, systolic murmur over the entire precordium, lungs clear to percussion and auscultation, and no significant changes in the abdomen except for a healed appendectomy scar. The voided urine was amber, murky, and acid. The specific gravity was 1.022, albumin was 3 plus, and sugar and acetone were absent. Microscopic examination disclosed many granular casts, an occasional leukocyte, and 20 to 30 erythrocytes per low-power field. A roentgenogram of the chest showed a diffuse fine mottling throughout both lungs with some hilar mottling. This appearance was interpreted as pulmonary edema. Blood counts and chemical determinations were not obtained. At 4:00 P.M., when 500 cc. of plasma and 1000 cc. of normal saline solution were injected intravenously, the pulse was rapid, irregular, and difficult to count. At 7:00 P.M. the patient was expectorating thick, yellow sputum, his breathing was stertorous and rapid, and he moved restlessly

in bed, although in coma. His pulse was 140 and his temperature was 98° F. At 1:30 A.M. on July 3rd, he died.

#### AUTOPSY FINDINGS

At autopsy, 8 hours after death, the body weighed 160 lbs. (78 kg.) and measured 72 inches (183 cm.) long. Externally, the only significant finding was a healed appendectomy scar. The panniculus adiposus was 3 cm. thick and the skeletal muscles were well developed and dark red. The peritoneal surface was smooth and moist except for adhesions of the omentum and the cecum to the under surface of the laparotomy scar. The pleural cavities and the pericardial sac were normal. The thymus was small and consisted of soft, yellow tissue. The thyroid gland *in situ* was normal in size, firm, gray-red, and finely lobulated. No tumors were found near the thyroid gland, although the parathyroid glands were not examined specifically.

The heart weighed 480 gm. and the epicardial fat was abundant. The myocardium was firm and gray-red. The right ventricle was 3 to 5 mm. and the left ventricle 16 to 20 mm. thick. A gray-red clot adhered to the lining of the left auricular appendage. The foramen ovale was closed. The valve leaflets were all thin and translucent. The valve circumferences were as follows: tricuspid, 12 cm.; pulmonic, 6.8 cm.; mitral, 10 cm.; and aortic, 6.5 cm. The coronary arteries were patent and their elasticity was moderately reduced. The first portions of the main branches showed irregular, yellow, intimal thickening. The aorta was moderately elastic and of usual caliber. A few pin-point, yellow dots marked the intima at the root and fine, yellow, longitudinal streakings were lightly scattered in the intima of the abdominal segment. The stems of the great vessels were patent.

The right lung weighed 780 gm.; the left lung, 810 gm. The surfaces were smooth and the lobes were discrete. Except for the apices and anterior margins, both lungs were subcrepitant on sectioning, dark red, finely black-streaked, and oozed plentiful foamy gray fluid. The trachea and bronchi had intact mucosa and a fairly abundant content of pale yellow mucoid material. The pulmonary arteries were normal. The mediastinal lymph nodes were small, soft, black and gray mottled.

The spleen weighed 420 gm. Visible through the thin capsule and extending down into the firm, flat, gray-red parenchyma were four dark purple, map-like areas, 1.5 to 4 cm. in greatest diameters.

The esophagus was normal. The stomach contained a scant amount of yellow, thin fluid and was lined by a reddened, well folded, intact mucosa. The small intestine contained similar fluid. The appendix was absent. The colon contained soft, well formed, dark brown feces.

The liver weighed 2,280 gm. The capsule was thin. The cut section was firm, tan, and focally marked by pale yellow areas, 6 to 12 mm. in diameter. The biliary ducts and blood vessels were well preserved. The gallbladder had a smooth serosa, a fairly thin wall, a dark green mucosa, and contained one large, oval stone measuring 4.5 by 3.5 by 2.5 cm., which was rough, tan and dark green, and six dark green, faceted calculi measuring 5 to 6 mm. in diameter. The bile was thick and dark green. The pancreas was normal in size, firm, tan, and coarsely lobulated.

The cortices of the large adrenal glands were fairly thick. The medulla of the right was entirely, and that of the left partly, liquefied.

The right kidney weighed 320 gm.; the left, 330 gm. The capsules stripped easily revealing smooth, swollen surfaces. The cut section (Fig. 1) was firm, flat, pale tan, and unevenly marked by pin-point, dark red dots. The cortices were 5 to 7 mm. thick and the striations were hazy. The pyramids were uniformly enlarged and the striations were fairly distinct. The pelves and ureters were normal. The urinary bladder was empty and the lining was gray-red and wrinkled. The prostate, seminal vesicles, testes and epididymides were grossly not remarkable.

The ribs and vertebrae were hard and difficult to saw. The marrow of the ribs was light tan, soft, and abundant. The cranium was not examined.

#### *Microscopic Examination*

The tissues were fixed in 4 per cent formaldehyde, cut at 6  $\mu$  from paraffin, and stained with hematoxylin and eosin.

The thyroid showed large follicles well filled with colloid and lined by low cuboidal epithelium. The stroma contained a few small groups of lymphocytes. Calcium was deposited beneath the endothelium of many capillaries and in the intima of several small and medium arteries and veins.

Calcium was deposited in many muscle fibers of the heart (Fig. 2). It was most abundant in the muscle of the septum and left ventricle, moderately plentiful in the left auricle, sparse in the right ventricle, and absent from the right auricle. Subendothelial calcium deposits were observed in blood vessels of all sizes and the degree of deposition paralleled that seen in the myocardium of the four chambers. Much calcium was deposited in the endocardium of the left auricle, the appendage of which was stuffed by loosely adherent ante-mortem thrombus. In the aorta the intima was mildly thickened by lipoids, small groups of foamy macrophages, calcium, fibrous connective tissue, and hyalin. The other coats were intact.

The lungs revealed the following changes: diffuse hyperemia, edema fluid distending most alveolar ducts and alveoli, numerous alveolar phagocytes, dust macrophages in alveoli and in small groups in the stroma, and deposition of calcium (Fig. 3) in abundance beneath the endothelium of veins of all sizes, in many alveolar walls, in the submucosa of many bronchi and bronchioles, and in small amounts in the media of some arteries.

The spleen showed engorged sinusoids, recent sinusoidal hemorrhages, demarcated areas of coagulation necrosis, and calcium deposits in the intima of many large and small arteries and veins. The accessory spleens contained a few blood vessels with slight intimal calcium deposits.

In the upper half of the fundic mucosa of the stomach the glands were greatly autolyzed. The lower half showed extensive deposits of calcium in and around glandular crypts (Fig. 4) with involvement of both interstitial tissue and gland cells.

The parenchymal cells of the liver showed the finely foamy appearance of abundant glycogen. A few groups of liver cells contained coarse cytoplasmic fat vacuoles. The blood vessels were well preserved and contained no calcium. The periportal areas and the biliary ducts were intact. The perimuscular coat of the gallbladder revealed increased fibrous connective tissue and infiltrations of lymphocytes.

In the pancreas, calcium was deposited beneath the endothelium of blood vessels of all sizes, including interacinar and islet capillaries. Calcium was present also in the lamina propria of several medium and large ducts. An area of acinar tissue and fat tissue was partly liquefied and infiltrated by many segmented neutrophils and monocytes. Several groups of acini and some islets were autolyzed.

The adrenal glands displayed no significant histologic abnormalities.

In the kidneys (Fig. 5) numerous masses of calcium were found in all segments of the tubular system of the nephrons and were intermingled with sloughed calcified epithelial cells in the lumina of the tubules. Hyaline droplets distended many epithelial cells lining the convoluted tubules. Recently formed thrombi, some focally organized, plugged many medium and large veins. Many lymphocytes infiltrated the interstitial tissue. Calcium was deposited beneath the endothelium of some large arteries. Granules and masses of calcium were seen in the capsular fluid of some glomeruli and foreign body giant cells surrounded a few masses of calcium in the tubules. Hyaline casts filled many tubules and leukocytic casts distended others. A few glomeruli were obliterated by fibrosis, but most were well preserved.

The urinary bladder was not remarkable.



The prostate contained a few groups of moderately dilated acini. In the peripheral part of the stroma some large arteries exhibited deposits of subendothelial calcium. A few spermia were present in the tubules of the testes. The seminal epithelium was developed through the spermatid stage. An occasional tubule was obliterated by fibrous connective tissue.

A section of rib (Fig. 6) revealed a marrow-cell/fat-cell ratio of 75/25, fairly numerous megakaryocytes, a great increase in the myeloid/erythroid ratio, and a definite shift to the left in the neutrophilic granulocyte line. The cortical and medullary bone was involved by extensive ragged fraying and thinning of the bony trabeculae with countless osteoclasts nestling in the recesses along the scalloped edges of the bony trabeculae. Giemsa staining confirmed these findings.

The final anatomic diagnoses were as follows: Metastatic calcification, on the basis of hypervitaminosis D and haliphagia (ἄλς, *salt*, + φαγεῖν, to eat), involving the heart (chiefly of the endocardium and myocardium of the left auricle and ventricle), the lungs (pulmonary veins, alveolar walls, and bronchi), the fundic mucosa of the stomach, the kidneys, the pancreatic ducts, and the blood vessels of the thyroid gland, heart, spleen, pancreas, and prostate; calcific and hyaline drop-let changes in the renal tubules with chronic interstitial inflammation and venous thrombosis; cardiac hypertrophy (480 gm.); mural thrombosis of the left auricle; pulmonary edema; acute passive congestion of viscera; recent infarcts in the spleen; osteoclastic resorption of bone; myeloid hyperplasia of bone marrow; acute focal pancreatitis; chronic cholecystitis and cholelithiasis; slight fatty metamorphosis of the liver; two accessory spleens; and absence of appendix, fibrous peritonitis, and healed laparotomy scar.

#### *Qualitative Chemical Analysis of Lungs, Kidneys, and Gastric Mucosa*

Fragments of lung (mainly alveoli in the periphery), kidneys (cortex and medulla), and fundic mucosa of the stomach were ground separately into a fine mush in a mortar. Microscopic examination of these tissues suspended in distilled water revealed amorphous material and fragmented cells but no crystals. When treated with a 50 per cent solution of hydrochloric acid, none of these tissues showed any macroscopic change. However, when 50 per cent sulfuric acid followed by 50 per cent hydrochloric acid was applied to the lung tissue, a grossly visible, violent evolution of gas bubbles was observed indicating the presence of a carbonate radical. The sulfuric acid evidently acted in some way to release calcium carbonate from the organic matrix of the lung, so that the hydrochloric acid was able to react with the carbonate

to produce carbon dioxide. This reaction was negative with the tissues from the kidney and gastric mucosa. When a 50 per cent solution of sulfuric acid was mixed with all three tissues, abundant formation of calcium sulfate crystals was seen histologically, thus demonstrating the presence of a calcium ion in all of them. When the kidney and gastric mucosa were treated with concentrated nitric acid and ammonium molybdate reagent and then heated, a yellow precipitate was formed indicating the presence of a phosphate radical in these tissues. With the same test the lung tissue was negative, for not only did no precipitate form, but the organic matter in the tissue was completely digested, so that a clear solution resulted. In summary, the qualitative chemical analysis of these organs showed that calcium phosphate was the salt deposited in the kidneys and gastric mucosa and that calcium carbonate was the salt deposited in the lungs.

### DISCUSSION

The case described is definitely an example of metastatic calcification as is easily proved by reference to the works of Askanazy,<sup>1</sup> Wells,<sup>2</sup> deSanto,<sup>3</sup> Grayzel and Lederer,<sup>4</sup> Wells and Holley,<sup>5</sup> and Herbert, Miller, and Richardson.<sup>6</sup> The heavy deposition of calcium in the endocardium and myocardium of the left chambers of the heart, in the pulmonary veins, bronchi, and alveolar walls, in the fundic mucosa of the stomach, in the kidneys, and in many arteries, capillaries, and even in veins, has been described by these authors. The places where acids are formed; namely, the lungs, the fundic mucosa of the stomach, and the kidneys, are organs in which the tissues are rendered alkaline when these acids are elaborated so that calcium deposition is favored.<sup>2</sup> The calcification of the endocardium and myocardium of the heart, of the arterial and capillary channels of the systemic circulation, and of the capillary and venous channels of the pulmonary circulation may also be explained on this basis, since the pH of the blood is higher in these tissues than in the venous side of the circulation, especially in the right chambers of the heart and in the pulmonary arteries. The ingestion by the patient of four inorganic salts containing sodium ions was a factor tending to increase tissue alkalinity. The deposition of calcium in the tissues was in the nature of a precipitation in previously healthy tissue cells and stroma, for the only degenerative change was the hyaline droplet alteration of the epithelial cells of the convoluted tubules of the kidneys and the only inflammatory infiltrations of significance were the interstitial infiltrations of lymphocytes and the leukocytic casts in some tubules of the same organs. This is in conformity with the original concept of Virchow,<sup>18</sup> who thought that the calcium

deposits in the stomach and lungs of his cases represented a direct calcification of the tissue by which lime salts penetrated and filled up the constituent parts of the organs involved.

Large doses of vitamin D in various forms have been employed to produce metastatic calcification in animals. Smith and Elvove<sup>9</sup> observed calcium deposits in the thoracic aorta, interalveolar septa, and kidneys of rabbits given irradiated ergosterol. Biochemical analysis revealed a progressive increase of serum calcium and the development of high inorganic phosphorus levels. In this study an elevated serum calcium and an increased serum phosphate level were necessary to produce appreciable tissue calcification, whereas with low or normal serum phosphorus values, calcium was not deposited in the tissues, even though the calcium of the blood was elevated. The authors thought that the salt concerned was calcium phosphate precipitated out in the tissues.

By giving viosterol to rats, Shelling<sup>10</sup> found that a greater susceptibility to hypercalcification of soft tissues resulted when the amount of phosphorus in the diet was increased.

With rats on an alkaline diet and large doses of oral calciferol, Gough, Duguid, and Davies<sup>11</sup> observed a heavy, quantitatively measured deposition of calcium in the kidneys, which was more pronounced than when the animals were on an acid diet. In excretion studies they found that the average urinary phosphorus was relatively low on an alkaline diet as compared to that on an acid diet. They also showed that phosphorus excretion was relatively decreased on both acid and alkaline diets by the calciferol.

In the experiments of Hess, Benjamin, and Gross,<sup>20</sup> the excessive administration of irradiated ergosterol caused a hypercalcemia in dogs fed a diet absolutely free of calcium. They thought that this excess calcium in the blood must come from the bones. The hypercalcemia was greatly reduced by the intravenous injection of sodium bicarbonate, after which a great excess of calcium and phosphorus was found in the lungs and kidneys and the calcium content of both urine and feces was definitely diminished. The histologic reaction in the bones of guinea-pigs given large doses of irradiated ergosterol was studied by Grauer,<sup>21</sup> who found in sections of costochondral junctions and long bones a resorption of bony trabeculae which were abundantly lined by osteoclasts, an increase in the size of the lacunar spaces, a thinning of the cortex, a proliferation of fibrous connective tissue in the marrow, and hemorrhage in the marrow cavities.

By applying the results of these experiments to the findings in the patient described, the vitamin D he ingested could have caused hyper-

calcemia,<sup>9,20</sup> phosphate retention,<sup>9,11</sup> and mobilization of calcium phosphate from the bones<sup>20</sup> as indicated by osteoclastic activity.<sup>21</sup> His diet was definitely alkaline through the ingestion of four sodium salts, which would favor the deposition of calcium salts in the soft tissues such as those of the kidneys.<sup>11</sup> An added source of phosphate further to augment the level of phosphate in the blood was available from the sodium phosphate taken by the patient. Finally, the sodium bicarbonate could have enhanced the calcium retention and favored an increase of calcium and phosphorus in such organs as the lungs and kidneys.<sup>21</sup>

No obvious tumors were observed near the thyroid gland. The absence of osteitis fibrosa cystica militated against the presence of a neoplasm in an aberrant parathyroid gland. The changes in the kidneys of patients with primary hyperparathyroidism have been described by Anderson.<sup>22</sup> The characteristic features include the following: interstitial fibrosis, interstitial, *mainly peritubular*, calcification, interstitial infiltrations of lymphocytes and plasma cells, cystic dilatation of tubules, thickening and sometimes calcification of the basement membranes of tubules, a relative absence of active glomerulitis or involvement of the epithelial cells of the tubules, sometimes formation of calculi and superimposed ascending infection, and obstruction and dilatation of tubules by the peritubular calcium deposits. Not only do these microscopic changes differ strikingly from the histologic features for the kidneys in my case, but the weight of the kidneys in the cases described by Anderson<sup>22</sup> was far below the tremendous weight (650 gm., combined) of the kidneys in the case reported. Both the gross and microscopic findings in the kidneys of the hitherto reported human cases of hypervitaminosis D<sup>14-17</sup> are much more nearly comparable to those in my case.

The mural thrombosis of the left auricle and the thrombosis of the renal veins were possibly on the basis of a high tissue alkalinity associated with a high content of calcium ions in the blood in these locations to hasten the clotting reactions. Askanazy's<sup>1</sup> first case of widespread involvement of the skeletal system by malignant melanoma and hemorrhagic nephritis also showed thrombosis of the renal veins. The cardiac hypertrophy may be explained on the basis of enlargement of uncalcified muscle fibers to compensate for those involved by calcium deposits. The recent infarcts of the spleen could have been caused by lodgment of emboli from the mural thrombus in the left auricle, although these emboli were not actually demonstrated. The deposition of calcium in the lamina propria of the pancreatic ducts has not hitherto been de-

scribed in cases of metastatic calcification. No explanation is obvious for the myeloid hyperplasia of the bone marrow.

### SUMMARY

Widespread metastatic calcification involving the heart, lungs, gastric mucosa, kidneys, pancreas, and numerous blood vessels of a male patient, 44 years of age, was associated with the ingestion of vitamin D and alkaline inorganic salts. The reasons for this extensive direct calcification of soft tissues in all probability included hypercalcemia, phosphate retention, mobilization of calcium phosphate from the bones brought about by osteoclastic activity, a high alkaline diet, an excess of ingested phosphate, and the enhancement of calcium retention by the intake of sodium bicarbonate. Qualitative chemical analysis demonstrated the presence of calcium carbonate in the lungs and of calcium phosphate in the gastric mucosa and kidneys.

Since this paper was submitted for publication, a case of metastatic calcification in a woman, 32 years old, has been reported by J. M. Bauer and R. H. Freyberg (Vitamin D intoxication with metastatic calcification. *J. A. M. A.*, 1946, 130, 1208-1215). This woman received large amounts of irradiated ergosterol, largely calciferol, for 1 year before death. At autopsy calcification of the kidneys, right knee joint, myocardium, endocardium of the left auricle, systemic arteries, lungs, dura, and subcutaneous tissue of the left ischial region was found. Also present were a chronic fibrous pneumonitis and a chronic duodenal ulcer.

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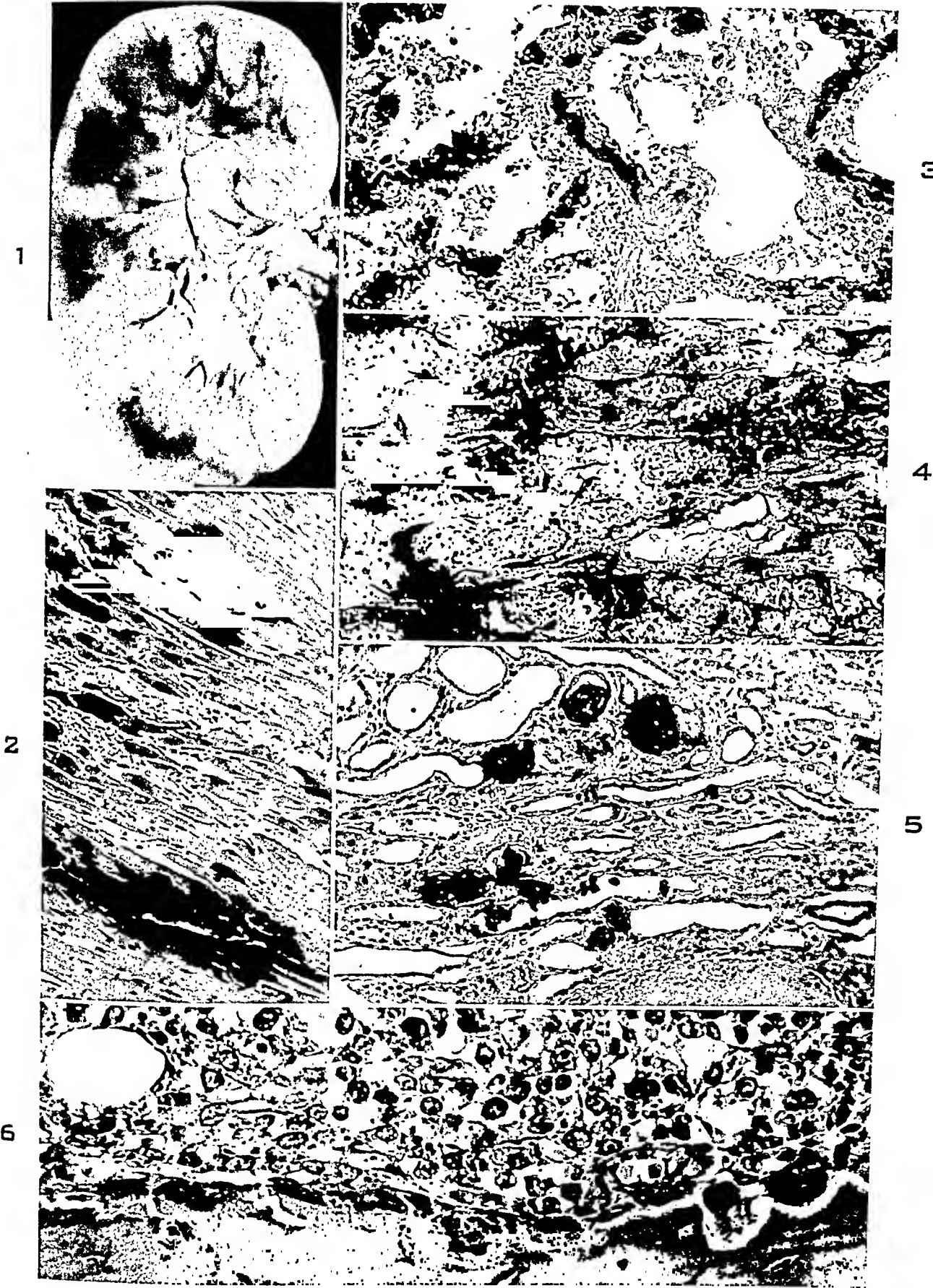
[ Illustrations follow ]

## DESCRIPTION OF PLATE

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### PLATE 269

- FIG. 1. Kidney, cut section. About  $\frac{1}{2} \times$ .
- FIG. 2. Myocardium of left ventricle, showing groups of calcified muscle fibers.  $\times 130$ .
- FIG. 3. Lung, showing calcium deposits in walls of alveoli.  $\times 130$ .
- FIG. 4. Gastric mucosa with calcium deposits in gland cells and interstitial tissue.  $\times 135$ .
- FIG. 5. Kidney, showing calcium masses in lumina of tubules, several with calcified cells. Of note is the renal vein in the lower right-hand corner, containing a thrombus.  $\times 130$ .
- FIG. 6. Rib with osteoclasts lining a ragged bony trabecula below, and cellular marrow above.  $\times 365$ .



Mulligan

Calcification Associated with Hypervitaminosis D





## PATHOLOGICAL CALCIFICATION IN THE GINGIVAE \*

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In a recent paper, Orban <sup>1</sup> has presented a type of gingival inclusion which he termed "dentin-cementum remnant." He believes that these rounded, basophilic, acellular calcified structures have their origin in fragmented dental structures. While Orban's explanation is direct and simple, it is believed that there is another method of formation of calcified gingival bodies. It is the purpose of this paper to show how calcified bodies may develop *in situ* because of local influences other than fragmentation of dental structures.

Material for this study was obtained from the slide files and reserve histological material that has been accumulated over a period of nearly 12 years by the University of Illinois College of Dentistry. Among 120 specimens of human gingivae, 4 showed lesions that were believed to be stages in the development of large and fully calcified bodies of the kind illustrated in Figure 8.

The following criteria were used in deciding which specimens were to be included in this study. (1) In order to exclude superficial débris, only the structures located beneath the epidermis were included. (2) To rule out cementicles, no structure in or close to the periodontal membrane was included. (3) Foreign bodies such as fragments of dental filling material were not included. While about one section in 25 of gingivae contained an inclusion, no idea as to the frequency of occurrence of such bodies in normal gingivae was obtained since the bodies were found only in granulation tissue that was usually old and hyalinized.

Pathological calcification in the gingivae undoubtedly occurs under the same conditions as it does in the other tissues. Barr <sup>2</sup> has summarized the local conditions which result in pathological calcification as: alkalinity of the tissue, the formation of phosphoric acid in the tissue, and the combination of calcium with protein. There are also general factors which may bring about pathological calcification: elevated parathyroid hormone levels, prolonged renal failure, and rapid bone resorption. Pathological calcification is therefore to be regarded as a biochemical process operating locally or generally which manifests itself by morphological changes that are frequently visible microscopically.

The first change observed in the formation of these calcified bodies was the transition of hyalinized connective tissues into rounded, homo-

\* Received for publication, November 6, 1945.

† Deceased.

geneous masses. This is shown in Figure 1. Such rounded masses already contain calcium (Fig. 2). Clumping or agglutination of these "spheroids" leads to the formation of the larger calcified masses (Figs. 2, 4, and 5). After the calcified masses agglutinate, they become more dense (Figs. 6 and 7). By examining only large, rounded, blue-staining masses such as are shown in Figure 8, it is impossible to tell whether they originated by fragmentation of dental structures and are "dentin-cementum remnants," or whether they came about by the slow deposition of calcium. But after examining earlier and smaller bodies, one is forced to conclude that such bodies originate by infiltration of calcium salts, rather than by fragmentation of teeth. That the structures in Figure 8 are not remnants of dental filling material is shown by two facts. There are structures of various sizes present in the immediate area representing different stages, and there is an internal structure visible under strong light.

Fibroblasts in long and intimate association with calcium salts may acquire bone-forming properties. Because of this metaplasia, bone is often seen in calcium deposits in the eye, the walls of arteries, and in old areas of inflammation. Usually the transformation of calcium deposits into bone is by endochondral ossification.<sup>3</sup> Bone probably forms in areas of pathological calcification of the gingivae, as occasionally imperfectly forming bone was found close to areas of calcified nodules (Fig. 9).

#### SUMMARY

There are, then, two views as to the origin of round calcified bodies such as those shown in Figure 8. Orban has explained them as resulting directly from a fragmentation of the dental structures; that is, as being "dentin-cementum remnants." The view set forth in this paper is that they result from calcified deposits in necrotic products of chronic inflammation of gingival tissues. If one examines only the end-product such as the large and fully calcified body (Fig. 8), it is impossible to determine whether it developed by fragmentation of dental remnants or by a process of pathological calcification. That the latter method of formation is possible is demonstrated in this study. It is also evident that ossification sometimes follows pathological calcification in gingival tissue.

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[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

### PLATE 270

All sections were stained with hematoxylin and eosin except Figure 2. (Photomicrographs by Dr. Merrill Shepro.)

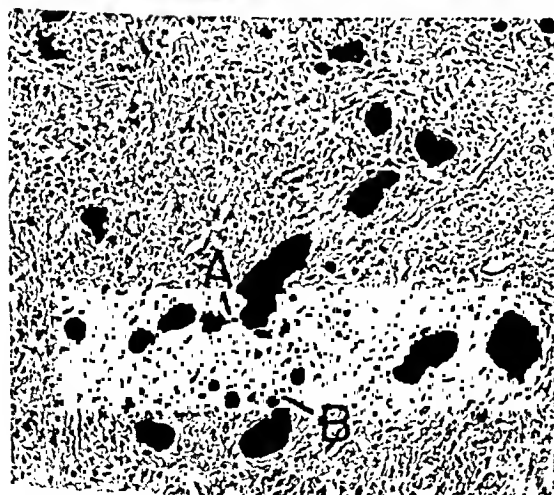
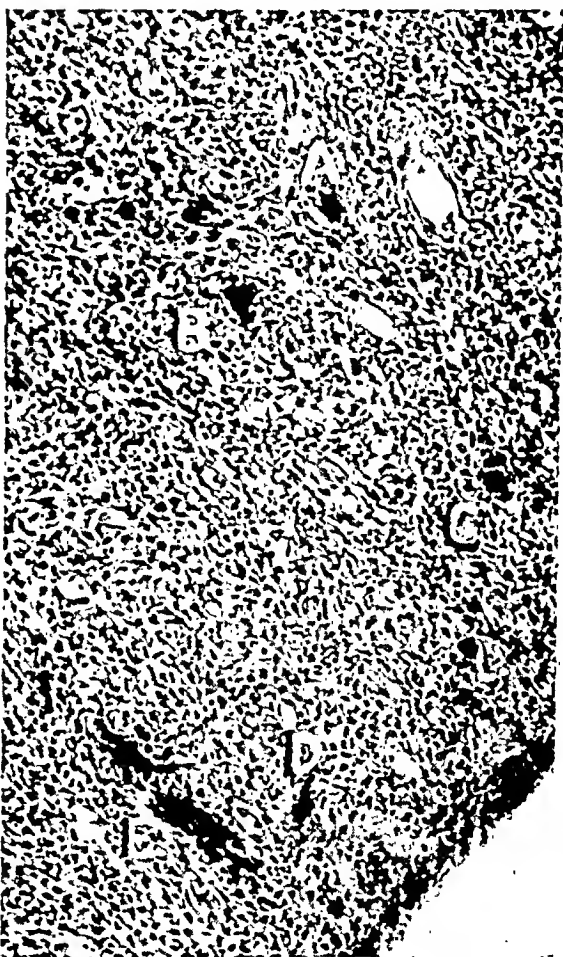
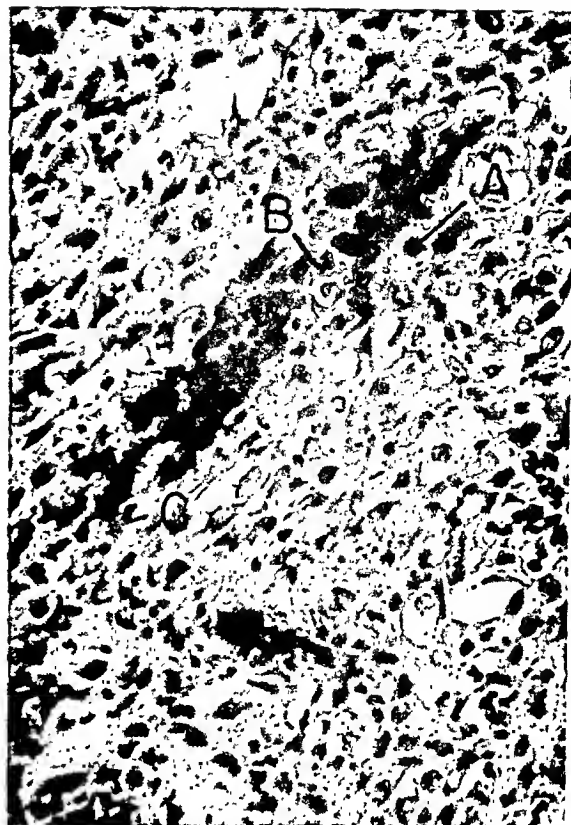
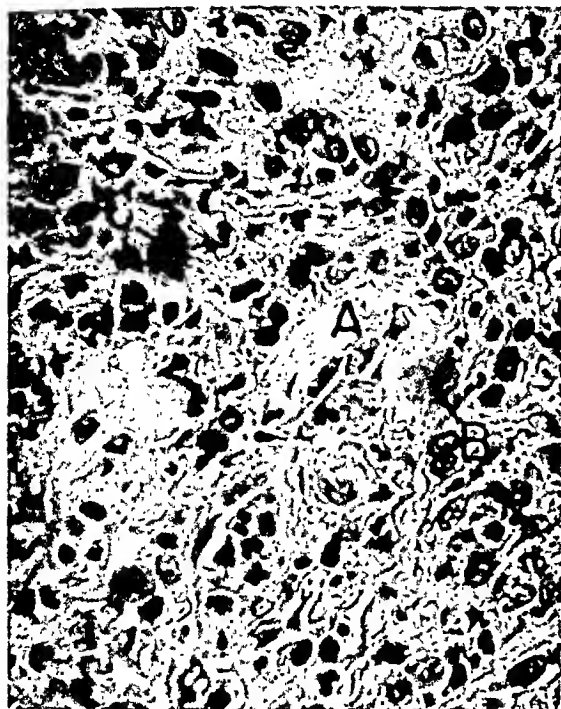
FIG. 1. Photomicrograph of gingivae showing the transition of dense hyalinized connective tissue, A, to spheroids of pathological calcifications, B and C.  $\times 490$ .

FIG. 2. Calcium is present during the spheroid stage, as shown by von Kossa's stain for calcium.  $\times 880$ .

FIG. 3. "Drawing together" or agglutination of the spheroids to form clumps. This is shown particularly at A and B.  $\times 100$ .  
Figures 1, 2, and 3 are from the same specimen.

FIG. 4. Clumping, A, B, and C, of calcareous bodies as shown in another specimen of chronically inflamed tissue.  $\times 650$ .

FIG. 5. Clumping results in basophilic rounding bodies shown at A, B, C, and D. E is the area shown in Figure 4.  $\times 200$ .



Barnfield

Calcification in the Gingivae

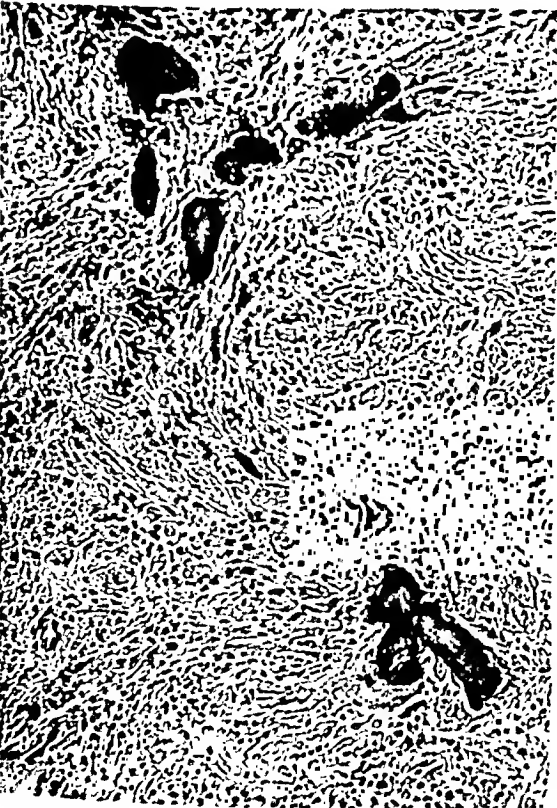
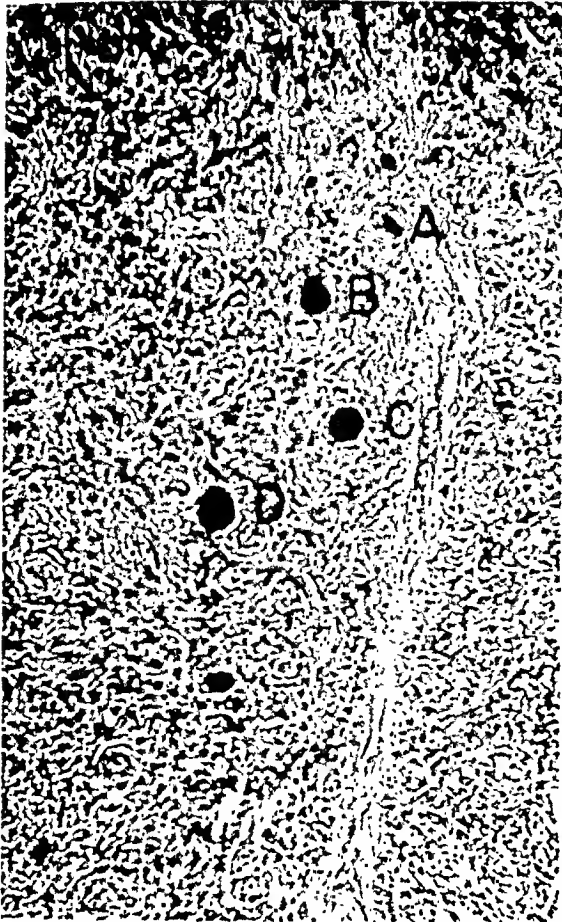
PLATE 271

FIG. 6. The rounding bodies become dense, as shown at A and B. Some have a concentric arrangement, C and D.  $\times 200$ .

FIG. 7. The pathological calcareous bodies here resemble bone. This tissue was taken from a site just opposite (on the anterior surface of the alveolar bone) that from which Figures 1, 2, and 3 were obtained.  $\times 300$ .

FIG. 8. The calcareous bodies become large and extremely dense. These have been termed "dentin-cementum remnants."<sup>1</sup>  $\times 500$ .

FIG. 9. Ossification sometimes follows pathological calcification. This area of osteogenesis was found in the same specimen, and close to, the bodies shown in Figures 4 and 5.  $\times 440$ .



Barnfield

Calcification in the Gingivae





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\* Abstract of paper presented at the meeting of The American Association of Pathologists and Bacteriologists held at Chicago, March 8 and 9, 1946.

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